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ERRATA AND AUTHOR'S EMENDATIONS

Page 25, line 12, "of" should be "or."
Page 32, eighth line from bottom, "Hybrid 133" should be "Hybrid 143."
Page 40, Table 2, fourth column, under "Dry matter," first line from bottom, "58.0" should be "58.8."
Page 40, Table 2, fifth column, under "Organic matter," first line from bottom, "58.8" should be "58.7."
Page 41, Table 3, fourth column, under "CO_3," line 16, "4,847.9" should be "4,847.8."
Page 50, Table 10, delete "eliminated" immediately following "0.707" in heading of table.
Page 55, Figure 1, in legend, "affcteed" should be "affected."
Page 84, Table 2, last column, delete "per day" in box head.
Page 174, line 9, "improved" should be "reduced."
Page 174, under "Tests in 1928," lines 8 and 9, "increased it 2 per cent for" should be "had no effect on."
Page 188, second line from bottom, "141" should be "161."
Page 189, line 11, "141" should be "161."
Page 258, line 6, "CO^2" should be "CO_2."
Page 398, Table 3, second column, under "Midseason," "U. S. D. A. No. 672" should be "U. S. D. A. No. 682."
Page 451, footnote 9, line 1, "5 gm." should be "0.5 gm."
Page 579, Table 2, third column, under "s," footnote reference figure "4" should be "3."
Page 579, footnote 4 (b), "$y=3.881+0.324\,x$" should be "$y=-3.881+0.324\,x$."
Page 700, footnote a to Table 4, "or" should be "of."
Page 768, line 4, "chlorate" should be "chloride."
Page 783, in legend for Figure 4, "×1,500" should be "×350."
Page 917, line 5, under "Botanical Description of *Bikukulla formosa*," "orolla" should be "Corolla."
Page 956, tenth line from bottom, "Berkfeld" should be "Berkefeld."
Page 1031, line 24, "Noxtoxus" should be "Notoxus."
Page 1047, paragraphs 1 and 2, immediately following Table 5, should be inserted immediately after Table 3, page 1044.
Page 1051, line 15, "Valles" should be "Vallese."

JOURNAL OF AGRICULTURAL RESEARCH

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BACTERIAL BLIGHT OF POPPY CAUSED BY BACTERIUM PAPAVERICOLA, SP. NOV.[1]

By MARY K. BRYAN, *Associate Pathologist, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture,* and FRANK P. MCWHORTER, *Pathologist, Virginia Truck Experiment Station*

INTRODUCTION

A striking disease of the Shirley poppy (*Papaver rhoeas* L.) was observed by the junior writer while visiting the Council plant farm, Franklin, Va., in May, 1927. Since the disease was decidedly destructive, probably of bacterial etiology, and apparently unlike any heretofore-described poppy disease, a rather full description of it was immediately sent to the Office of Mycology and Disease Survey of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C. The present paper is a preliminary report intended to establish the bacterial nature of the disease and to describe the pathogene as a new species of Bacterium.

HISTORY OF THE DISEASE IN THE TYPE LOCALITY

Through visits to the garden of the Council plant farm, Franklin, Va., and by correspondence with its owner, the writers are able to present the following brief history of the garden plot from which the type material was collected.

Seeds of the Shirley poppy were planted in the spring of 1926. According to the grower, a few of the plants developed the disease during the latter part of the first (1926) season; at that time the disease was destructive to the individual infected plants, but not to the plot as a whole. The 1926 plants reseeded the area and served as a source for the 1927 crop. The 1927 plants, when small, were given a generous application of sheep manure, which, according to the grower, may have predisposed the plants to the disease, for during the 1927 season it was conspicuous even before the plants reached the flower-bud stage. By the middle of the blooming season many of the plants were killed, and those remaining were made unsightly by numerous black spots on leaves and buds. While the plants were in this condition the owner burned over the area, hoping to eradicate the disease and to prevent the poppies from reseeding. Some escaped, however, for in 1928 a few came up; these were left for a while, but since they developed the disease long before blooming time, they too were burned.

Type material was collected from this plot by the writers in May, 1927, and later filed in the herbarium of the Office of Mycology and Disease Survey.

[1] Received for publication Apr. 29, 1929; issued January, 1930. This paper resulted from a joint project carried out by the Virginia Truck Experiment Station, Norfolk, Va., and the Bureau of Plant Industry, U. S. Department of Agriculture.

IMPORTANCE AND GEOGRAPHICAL DISTRIBUTION

The behavior of this disease on poppies at Franklin, Va., points to its having great possible economic importance. It not only made the plants unsightly by conspicuous black spotting of the leaves and stems but also killed many of them. Moreover, later infection tests have shown that it is readily transmissible.

The writers have observed this disease on the Shirley variety at the Franklin locality only. That it has occurred elsewhere in the United States is shown by a report of Clinton [2] in 1909 and by herbarium material kindly furnished by him. Clinton's collection of the leaf spot form of the disease was made at Westville, Conn. In appearance, the lesions are identical with those on the Virginia specimens. Bacteria were found in great abundance in the spots, but, as was expected, attempts to isolate them from this herbarium material failed.

In addition to the Shirley poppy, the Oriental poppy (*Papaver orientale* L.) must be considered as susceptible. Mature pods and leaves of Oriental poppies with lesions similar to those on Shirley poppy were collected at North, Va., by the junior writer in June, 1927. The organism isolated from these leaves showed slight cultural variations from the Shirley poppy strain, but when inoculated into Shirley poppies it produced lesions characteristic of the disease.

SYMPTOMATOLOGY

The following discussion is based on the examination of plants naturally infected in the type locality and on observation of plants subsequently inoculated with pure cultures of the organism. Lesions were abundant on all the aerial parts, but for convenience in discussion the symptoms on leaf, stem, and floral parts are presented separately.

EFFECT ON LEAVES

The general appearance of infected leaves is shown in Figures 1 and 2. The intensely black spots suggest those produced on rose leaves by *Diplocarpon rosae* Wolf.

Young infections are first evident as minute water-soaked areas; these soon darken into definite spots bounded by a hyaline or water-soaked ring. This ring is seldom evident in older spots.

The size and the shape of mature spots are affected by the distribution of the primary lesions on the leaf. When scattered they are distinctly circular, clearly defined, often zonate (fig. 3), and may attain a diameter of 3 to 4 mm. Eventually the tissues between the spots yellow and turn brown, but the individual spots remain distinguishable by their darker color and zonate markings. The zonation is comparable to that developed in wildfire of tobacco and bacterial leaf spot of Delphinium. When the infections occur close together, the individual spots are small (1 mm. or less in diameter), but by coalescing they form large black or sepia-black areas. A slimy bacterial exudate is noticeable at times on the lesions. Leaf infections weaken the leaves to such an extent that defoliation results.

[2] CLINTON, G. P. REPORT OF THE BOTANIST. Conn. Agr. Expt. Sta. Bien. Rpt. 1907-08 (pt. 12): 870, 1909.

Entrance of the bacteria is accomplished through the stomata. (Pl. 1, B.) Sometimes the organism enters the veins from the intercellular spaces or through the water pores at the tips of the serratures, so that the disease becomes systemic. When this occurs the veins darken and the surrounding tissues yellow and deaden, thereby assuming the appearance shown in Figure 4. This effect suggests the behavior and appearance of water-pore lesions typical of black rot on cabbage, but cross inoculations between *Bacterium campestre* and the poppy organism were negative.

FIGURE 1.—Shirley poppy leaf showing isolated spots of infection. About twice natural size

EFFECT ON THE STEMS

The lesions on the stems are intensely black, as are those on the leaves (fig. 5); but, unlike the leaf lesions, the stem lesions tend to elongate longitudinally. When close together they may coalesce and girdle the stems. They are confined chiefly to the cortical regions, but the girdling process is sufficiently severe to cause the smaller plants to break over.

EFFECT ON FLORAL PARTS AND PODS

All floral parts are susceptible, but the young sepals are especially so; these may be partially or entirely blackened. (Fig. 5.) The organism is able to pass through the sepals and attack the young petals beneath. When this occurs further development of the flowers is stopped.

The spots on the pods (fig. 5), like those on the leaves, are black and conspicuous, but the margins are more promi-

nently water-soaked. The slimy appearance of the spots, referred to above, is more noticeable on the pods than on any other part of the plant.

FIGURE 2.—Shirley poppy leaf with numerous scattered spots of infection and large dead areas formed by coalescing spots. Natural size

GENERAL EFFECT ON THE PLANT

The general effect on the plants has a twofold economic significance. In the first place, the conspicuous black leaf spots make the plants unsightly; and secondly, many of the plants are killed. The killing is caused in part by the girdling of the stems, in part by defoliation, and in part by the toxic or mechanical effect produced by masses of the organism when the disease becomes systemic.

PREVIOUSLY REPORTED BACTERIAL DISEASES OF POPPY

The only report of bacterial blight of poppy, previous to that made by the junior writer, is that of Clinton.[3] Other bacterial diseases of poppy have been reported as noted below.

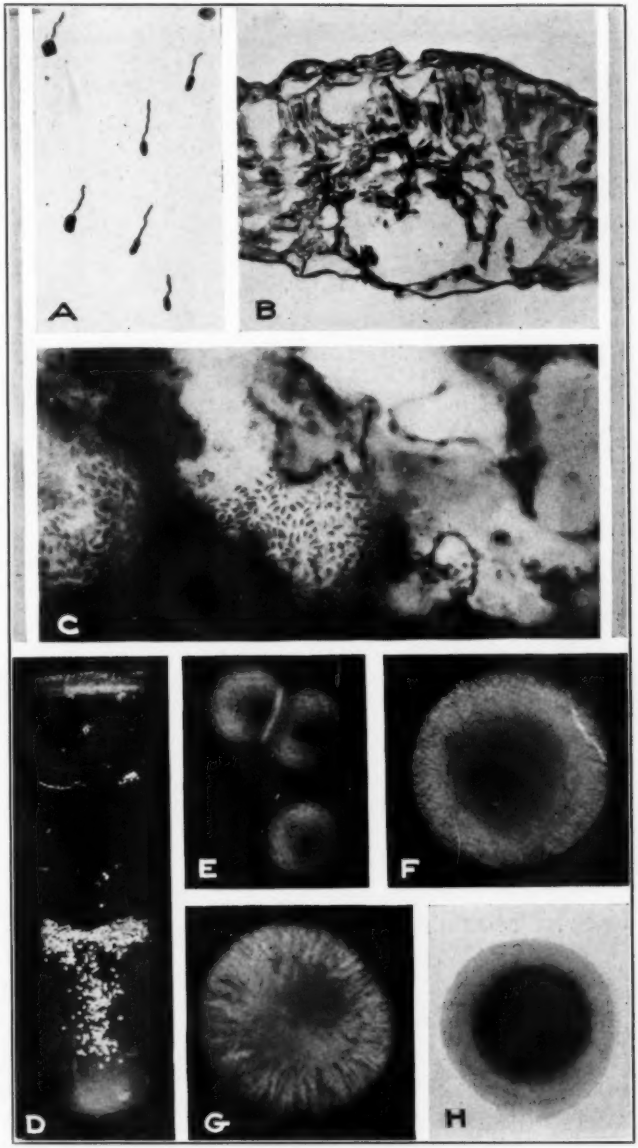
Marchal and Foex[4] in 1915 described a blight of the sepals and petals of poppy, but they did not designate the species from which they isolated a white bacterium similar to *Bacillus coli communis*. They did not report any inoculations with the organism that they isolated. The organism described in the present paper produces a yellow pigment.

In 1918 Hutchinson[5] reported from India a poppy disease which he considered to be due to bacteria. In describing it he stated: "Blackening and slimy decay of the stem and leaves of the opium poppy and other varieties was found to be due to bacterial

[3] CLINTON, G. P. Op. cit.

[4] [MARCHAL, P., and FOEX, E.] RAPPORT PHYTOPATHOLOGIQUE POUR L'ANNÉE 1913. Ann. Serv. Epiphyties 2:63-64. 1915.

[5] HUTCHINSON, C. M. AGRICULTURAL BACTERIOLOGY. India Bd. Sci. Advice Ann. Rpt. 1916-17: 57-66. 1918.



A.—*Bacterium papavericola* showing flagella. × about 1,100.
B.—Section of inoculated leaf showing stomatal infection. × 200.
C.—Section of diseased leaf showing bacteria in the tissues. × 1,150.
D.—Milk culture 1 month old, showing masses of tyrosin crystals.
E–H.—Colonies on beef-infusion agar plates. × 5. Colony E, 3 days old; colonies F, G, and H, 5 days old. Colonies E, F, and G by oblique transmitted light; colony H by direct transmitted light.

rot; a description of the disease and of the causative organism is in hand for publication." It appears that Hutchinson did not publish his data but instead turned them over to Ram Ayyar,[6] as reported below.

In 1927 Ram Ayyar [7] described from Pusa, India, a bacterial soft rot of garden poppy which is apparently that referred to by Hutchinson. The disease and the causal organism described by him are different from those recorded in this paper. The Indian disease found on *Papaver somniferum* at Cawnpore by Hutchinson later developed on *P. rhoeas* at Pusa, and the latter species was used in inoculation experiments by Ram Ayyar. Ram Ayyar made no reference to leaf spotting, but instead he described the disease as a soft rot of the stem and the organism as a peritrichiate, rod-forming, white or bluish-white growth on various culture media. The bacterial-blight organism of the present paper forms a yellow pigment and produces no soft rot.

FIGURE 3.—Isolated spots of infection on Shirley poppy leaf, enlarged to show zonate character. × 5

ISOLATIONS AND INOCULATIONS

The organism was found abundantly in old and young lesions on both species of the poppies studied. (Pl. 1, C.) It was readily isolated from fresh material by the poured-plate method, and subcultures from single colonies were used in inoculation experiments.

On June 20, 1927, a group of Shirley poppy plants in a garden, the only ones then available, were sprayed with a suspension of the pathogene isolated on June 2 from Shirley poppy. A gentle rain which continued during the evening provided sufficient moisture so that it was not necessary to cover the plants with inoculation cages. Infections were evident on stems and leaves on June 25.

Pure-culture reisolations made on June 28 from spotted leaves on these inoculated plants were used on September 21 to inoculate plants of Shirley and Oriental poppies by spraying. These plants were kept moist in inoculation cages in the hothouse for 48 hours. The Shirley poppies used were blooming plants, whereas the Oriental

[6] RAM AYYAR, C. S. A BACTERIAL SOFT ROT OF GARDEN POPPY. India Dept. Agr. Mem., Bact. Ser. 12: 29-33, illus. 1927.
[7] RAM AYYAR. C. S. Op. cit.

ones were seedlings with only a rosette of leaves. All were vigorous. Successful infections were thus obtained on the leaves, buds, and seed pods of the Shirley poppy and on the leaves of the Oriental poppy. On October 7 the pathogene was reisolated from all parts of these infected plants.

Poured-plate isolations from spots on Oriental poppy leaves collected at North, Va., were also used on September 21 to inoculate both species of poppy. The same method was used as with the isolations from the Shirley poppy. Infections resulted on both species, and successful reisolations were made.

FIGURE 4.—Systemic infection on leaf of Shirley poppy. Natural size

Good infections were also obtained when Shirley poppy plants in an outdoor plot were sprayed with the pathogene and kept moist under large flower pots.

The Shirley poppy appears to be more susceptible than the Oriental poppy, judging by the number of infections resulting from these series of inoculations.

THE PATHOGENE

MORPHOLOGY

Bacterium papavericola, sp. nov., is a short rod, 1 to 1.7μ long by 0.6 to 0.7μ wide, motile by means of a single polar flagellum. (Pl. 1, A.) It occurs singly, in pairs, or in short chains. Capsules occur in old agar cultures and in the rims and pellicles of bouillon cultures. No spores have been observed.

STAINING REACTIONS

The organism is decidedly Gram-negative and is not acid-fast. It stains readily with the usual bacterial stains.

CULTURAL CHARACTERS

Unless otherwise stated, the descriptions here given are based on cultures of the Shirley strain, but they are true also for those of the Oriental strain. Slight differences in amount rather than character of growth have been disregarded.

BEEF-AGAR PLATES.[8]—Colonies are visible on the second day, and by the third day they are 2 to 3 mm. wide, mustard yellow,[9] smooth, convex, round, finely crosshatched by transmitted light, translucent, margin entire. (Pl. 1, E.) Radiating lines are sometimes conspicuous (pl. 1, G) and at other times entirely lacking. (Pl. 1, F.) Colonies may attain a width of 7 mm. and often are slightly umbonate, the thinner margin showing as a paler yellow ring by transmitted light. (Pl. 1, H.) Secondary growths of paler color, in the form of

[8] Unless otherwise noted, all beef media used were made with beef infusion and peptone and had a pH of 6.8 to 7.0.

[9] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

wedges on the margin or circular areas in the body of the colony, are common in old colonies. A second type of colony sometimes appears; these are smaller, thinner, and deeper yellow. Colonies of the Oriental strain differ from those of the Shirley strain in internal markings only. They occasionally show fine concentric lines in the margins, but they are often finely granular throughout without other markings.

BEEF-AGAR SLANTS.—Growth is filiform, and scanty at first, becoming moderate but never heavy; it is translucent, smooth, finely granular or crosshatched, butyrous in young cultures, becoming viscid when old. The color, at first mustard yellow,[10] becomes primuline yellow with age (2 weeks). Numerous small, circular, secondary growths of paler yellow appear in old cultures.

The growth on slant cultures of the Oriental strain is consistently deeper yellow (primuline to light cadnium) than on those of the Shirley strain.

BEEF BROTH.—Clouding is prompt, and by the second day a pale-yellow rim and often an incomplete pellicle floating unattached to the rim have formed. In undisturbed cultures the pellicle becomes heavy and may or may not connect with the rim. Numerous small rectangular crystals form in the pellicle.

Growth on beef-extract agar and broth does not differ from that on agar and bouillon made with beef infusion.

POTATO CYLINDERS.—Growth is heavy on potato cylinders, covering the whole surface of the potato and filling the water with a dense, yellow slime. The diastatic action is so strong that the cylinder gradually shrinks until almost completely submerged in the yellow bacterial growth, which may eventually take on a brown tinge. Minute brown, spherical aggregates of crystals form in abundance between the growth and the side of the tube in old cultures. Cultures of the Oriental strain are indistinguishable at first from those of the Shirley strain. Eventually the growth becomes a decidedly deeper yellow and less abundant than in the Shirley strain, and the diastatic action is less vigorous.

THAXTER'S POTATO-DEXTROSE AGAR.—Growth on slant cultures is very abundant, pale yellow, and homogeneous. Color deepens with age to primuline yellow. The Oriental strain remains paler than the Shirley one on this medium.

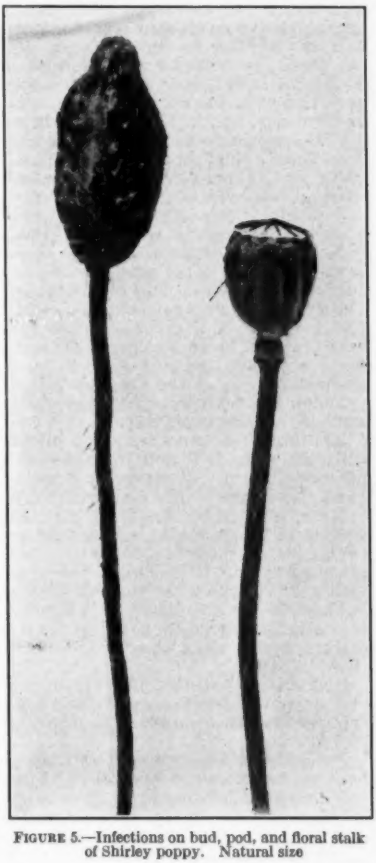


FIGURE 5.—Infections on bud, pod, and floral stalk of Shirley poppy. Natural size

SYNTHETIC MEDIA.—In Cohn's solution growth may not occur, and at best it is almost imperceptible. Fermi's solution clouds weakly but develops no fluorescence. In Uschinsky's solution clouding is slow, becoming moderate but without fluorescence.

PHYSIOLOGICAL CHARACTERS

LIQUEFACTION OF GELATIN.—In beef-infusion gelatin held at 17° to 20° C. liquefaction begins in two or three days; it is saucer shaped at first, but later it becomes stratiform. It proceeds so slowly that after two weeks only one-half

[10] RIDGWAY, R. Op. cit.

is liquefied. Liquefaction is not complete even when the cultures are held for six weeks. The liquefied portion is clear, with a yellow pellicle and abundant sediment.

LIQUEFACTION OF BLOOD SERUM.—Liquefaction, which is preceded by clearing of the medium, begins by the third day; clearing and liquefaction proceed so slowly that in three weeks only one-half of the medium is liquefied.

FERMENTATION OF SUGARS.—Various sugars and alcohols were tested for fermentation. On beef-extract agar slant cultures with bromcresol purple as an indicator, acid was evident on the third day from saccharose, dextrose, and galactose. The yellow color progressed slowly to the bottom of the tubes. On the fourth day an alkaline reaction appeared in the upper end of all these cultures, and by the fifteenth day the agar was alkaline throughout in all. At no time was there any evidence of acid formation from levulose, lactose, maltose, glycerin, or mannit in this agar. When phenol red was used as an indicator in the same agar and with the same sugars, an alkaline reaction was evident on the second or third day, and in 10 days the whole of each culture was alkaline.

The organism was tested on the same carbohydrates in synthetic agar made according to the formula in the Manual of Methods [11] with bromcresol purple as indicator. There was no trace of alkali at any time in these cultures. An acid reaction was evident in saccharose and dextrose on the second day, on the fifth day in glycerin, and on the third or fourth day in the others. Within three weeks all the cultures were acid throughout.

No gas was formed from these sugars and alcohols in fermentation-tube cultures containing 1 per cent peptone and 2 per cent of the sugar. Clouding was confined to the open ends of the tubes.

HYDROLYSIS OF STARCH.—The starch of steamed-potato cylinders is so completely destroyed that the shrunken cylinders are almost or completely submerged in the mass of bacterial slime. On starch-agar plates streaked with the organism, an area 25 mm. wide is cleared beyond the growth within seven days. The diastatic action of the Oriental strain is decidedly less vigorous. The potato cylinders are never completely submerged. On starch-agar plates the clear area after growth for seven days is only 5 to 8 mm. wide.

REDUCTION OF NITRATE.—No nitrate reduction was evident in nitrate-broth cultures, when 4, 7, and 10 days old, by using the starch-potassium-iodide sulphuric-acid test. However, a weak but decided nitrite reaction was obtained at all these ages by the α-naphthalamine sulphanilic-acid test.

REACTIONS IN MILK.—Separation begins on the second day with a layer of whey 1 to 3 mm. deep above a fluid curd. In litmus milk there is a slight reddening on the third day, followed immediately by reduction of the litmus, which is completed in 4 to 10 days. Color begins to return 3 or 4 days later. Peptonization is evident on the third or fourth day; clearing proceeds slowly downward in bands and is completed in two weeks. In cultures 1 month old the curd is a coarsely flocculent, fluid, translucent mass occupying the lower half of the culture, with a clear whey above, holding numerous masses of tyrosine crystals. (Fig. 4.)

Reduction of methylene blue in milk begins on the third day and is completed on the fourth day. Coagulation, peptonization, and the formation of tyrosine crystals proceed as in litmus milk. Old cultures (1 month) are orange buff in color.

PRODUCTION OF AMMONIA.—Ammonia production is strong, as indicated by tests made with strips of filter paper wet with Nessler's solution and inserted in beef-broth cultures 8 and 10 days old.

PRODUCTION OF HYDROGEN SULPHIDE.—Lead-acetate paper tests for the presence of hydrogen sulphide in beef broth gave on the third day a distinct positive reaction which became strong by the sixth day.

PRODUCTION OF INDOL.—No indol was formed in Dunham's solution within 15 days. *Bacillus coli communis* used as a check gave the usual positive result.

TOLERATION OF SODIUM CHLORIDE.—The organism grows readily in beef broth containing 1.2 or 3 per cent sodium chloride. Growth is retarded by 4 per cent and inhibited by 5 per cent.

RELATION TO FREE OXYGEN.—In agar stabs the best growth occurs at and near the surface; no growth occurs in the bottom of the stabs, or in stabs which have been covered immediately by 10 c. c. of agar. There is no growth in the lower part of shake agar cultures. Growth is confined to the open end of fermentation tubes.

[11] SOCIETY OF AMERICAN BACTERIOLOGISTS. COMMITTEE ON BACTERIOLOGICAL TECHNIQUE. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA, FOR USE WITH THE DESCRIPTIVE CHART OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS. 48 p., illus. Geneva, N. Y. [1928.]

OPTIMUM pH FOR GROWTH.—In a series of beef broths ranging from pH 4.4 to 9.6, clouding occurred within 24 hours in pH 6.1 to 7.7, but it was heaviest between pH 6.9 and 7.6. By the second day all were clouded between pH 5.6 and 8.3. No growth took place beyond these limits.

RESISTANCE TO DESICCATION.—Sterile cover glasses wet with a loop from 24-hour-old cultures were placed in sterile Petri dishes and kept dry in the dark. At intervals covers were dropped into beef broth. After drying for four months and at intervals before, the bacteria clouded the bouillon promptly.

In herbarium specimens the bacteria were alive after drying for 20 months. On plates from these dry leaves colonies came up promptly and appeared as vigorous as those from fresh material; they were proved infectious by inoculation on Shirley poppy leaves.

TEMPERATURE RELATIONS.—The thermal death point is 52° C. Best growth occurs between 25° and 30°. The maximum temperature for growth is about 35°.

TECHNICAL DESCRIPTION

Bacterium papavericola, sp. nov.

A short rod, 1 to 1.7 μ long by 0.6 to 0.7 μ wide, motile by means of a single polar flagellum, occurring singly, in pairs, or in short chains. It is capsulate but forms no spores; is Gram-negative and not acid-fast; forms round, bright-yellow colonies on beef agar; clouds bouillon and forms a rim and a pellicle; diastatic action strong; liquefies gelatin rather slowly; forms acid without gas from dextrose, saccharose, galactose, levulose, lactose, maltose, glycerin, and mannit; reduces nitrates; causes weak coagulation and complete peptonization in litmus milk with reduction of litmus and the formation of tyrosine crystals; produces ammonia and hydrogen sulphide but no indol; is aerobic; grows best in a pH of 6.9 to 7.6; withstands drying for 4 months on cover glasses and for 20 months or longer in herbarium specimens; optimum temperature is between 25° and 30° C.; causes black spots on all aboveground parts of the Shirley poppy and on leaves and pods of the Oriental poppy.

SUMMARY

Bacterial blight of poppy is a disease which attacks Shirley and Oriental poppies. It produces unsightly black spots on leaves, stems, buds, and seed pods; it spreads rapidly and when severe may kill the plants.

The disease has been found in Virginia and Connecticut. The organism causing it has been isolated and successfully inoculated into Shirley and Oriental poppies. *Bacterium papavericola* is presented as the name for the pathogene. A description of the cultural and physiological behavior of *Bact. papavericola* is given.

SPECIES OF THE NEMATODE GENUS STRONGYLOIDES PARASITIC IN DOMESTIC SWINE[1]

By BENJAMIN SCHWARTZ, *Senior Zoologist*, and JOSEPH E. ALICATA, *Junior Zoologist, Zoological Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The object of this paper is to clear up the existing confusion and uncertainty with reference to the specific identity of the nematodes of the genus Strongyloides parasitic in domestic swine. The name *Strongyloides suis* has been used by certain workers to designate a distinct zoological species, and by others it has been used merely as a convenient host designation for Strongyloides from swine. Probably the majority of helminthologists who have expressed an opinion on this subject have regarded *S. suis* as a synonym or as a probable synonym of *S. papillosus* of sheep. The investigations reported in this paper, together with a study of the literature relating to Strongyloides of swine, have shown that two species of this nematode genus have been reported from these host animals. One species, which is the form involved in an extensive account of the pathogenicity of Strongyloides to young pigs by Reisinger (*14*),[2] is considered in this paper as *S. suis*, and the other species, which presumably is the form which many helminthologists have considered identical with *S. papillosus*, is regarded as new.

REVIEW OF LITERATURE

Reisinger (*14*), Sandground (*15*), Baylis (*1*), and other workers who have studied Strongyloides have overlooked the fact that Pagenstecher (*9*) recorded Strongyloides from pigs, presumably for the first time, and that his observations antedated those of Grassi (*3*) and Lutz (*7*). Lutz has erroneously been credited with being the first to report Strongyloides from swine. Pagenstecher (*9*) records small, slender worms, consisting entirely of female specimens, from the small intestines of pigs. He recognized these worms as related to Anguillula (synonym, pro parte, of Strongyloides) and described their morphology in considerable detail, listing all the salient generic characters of the genus Strongyloides. Pagenstecher says that the ovary is short in young specimens and that it increases in size as the worms grow older, finally becoming twisted in loops around the intestine, anteriorly and posteriorly, a character which has been figured and described with reference to Strongyloides of swine by later workers.

Grassi (*3*) reports that animals other than man harbor *Anguillula intestinalis* (as used by Grassi this name is a synonym of *Strongyloides stercoralis*, parasitic in man); he records these parasites from swine, rabbits, and weasels, and he also refers to their occurrence in cows, chickens, cats, sparrows, and mice, and notes their absence in several species of amphibians and reptiles which he examined. He states

[1] Received for publication May 10, 1929; issued January, 1930.
[2] Reference is made by number (italic) to "Literature cited," p. 22.

that the forms from the different hosts differ in certain respects, especially in size, and expresses the opinion that they belong to the same genus.

The same author (*4*) used the name *Rhabdonema longus* for the form from sheep and reiterates the opinion that the same species also occurs in the rabbit, the weasel, and the pig. He says that the parasitic female is 7 mm. long and notes that in the free-living generation of *R. longus* the females outnumber the males to the extent of 1,000 to 1. He also notes that the free-living females of this species often die without having copulated.

Lutz (*7*), apparently unaware of previous records of the occurrence of Strongyloides in pigs, reports observations on these nematodes from swine, based on a study of the parasitic females which he obtained on necropsy from a pig in Brazil, as well as observations on the free-living males and females which he cultured in the feces obtained from the same host animal. Lutz says that he recognized the similarity of these forms to those of *Anguillula stercoralis*, from man, as described by Perroncito (*10*), noting, however, certain differences in size. He states that the parasitic females from the swine are about 1 cm. long and that the free-living males and females, which he obtained from fecal cultures in approximately equal proportions, are twice the size of those cultured from human feces. He also notes that the eggs of the pig Strongyloides hatch outside of the body, whereas those of the human form hatch before they are eliminated with the feces.

Von Linstow (*6*) uses the name *Strongyloides suis* for the first time and erroneously credits Lutz with being the author of this name. Von Linstow not only proposed a new name, but at the same time that he proposed it he also relegated it to the synonymy, as he lists *S. suis* as a synonym of *S. longus* (Grassi and Segré) and gives the following hosts for this species: *Ovis aries*, *Sus scrofa*, *Lepus cuniculus*, *Foetorius vulgaris*, and *Mus decumanus*. Aside from listing the hosts of *S. suis* and stating that it is 6 mm. long, he gives no description. *S. longus* (Grassi and Segré) is regarded at the present time as a synonym of *S. papillosus*.

Perroncito (*10*) reports Strongyloides from swine, especially from young pigs, and gives an account of the pathogenicity of these parasites. His paper contains no data with reference to the morphology of the worms, which he does not name specifically.

Ransom (*13*) reports for the first time, the occurrence of Strongyloides in pigs in the United States. He makes the following statement with reference to the specific identity of Strongyloides from American swine:

> The parasite of the pig, which has been identified as *Strongyloides longus* (=*S. papillosus*) by European observers, is probably *Strongyloides suis*. This species has been found a number of times in pigs at the Bureau of Animal Industry Experiment Station, Bethesda, Md.

Railliet (*11*) lists *Strongyloides suis* (Leuck.) from swine. The present writers have been unable to trace any reference to the specific name *suis* used by Leuckart in combination with any synonyms of the genus Strongyloides.

Reisinger (*14*), in an extensive paper on the pathogenicity of Strongyloides to young pigs, gives very valuable clinical data and figures and describes a species of Strongyloides for which he uses the

names *S. suis* and *S. longus* interchangeably. As will be shown later, the morphology of the worms as figured and described by Reisinger differs in several important respects from that of the worms which have been collected from pigs in Bethseda, Md., and studied by the writers.

Marotel (*8*) describes a chronic enteritis in pigs due to *Strongyloides suis*. His paper is not available to the writers. The abstracts of this paper which have been consulted contain little information in regard to the morphology of the worms, the parasitic female being described only as from 3 to 4 mm. long by 30μ to 40μ wide.

Fiebiger (*2*) regards Strongyloides from pigs as identical with *S. longus* of sheep and rabbits. His figure of this species from pigs agrees with Reisinger's figure of *S. suis*.

Hall (*5*) recognizes *Strongyloides suis* as a valid species, listing *S. longus* (pro parte) as its synonym, and noting for the first time that the forms found in the eastern part of the United States do not agree with the description of *S. suis* as given by European helminthologists.

Sandground (*15*) questions the validity of *Strongyloides suis* and states that he examined several specimens (parasitic females) of Strongyloides from swine obtained from the helminthological collection of the Bureau of Animal Industry, and found that the ovary is twisted around the intestine, as in *S. papillosus*. He says:

> The only possible distinction that could be made between these specimens and *S. papillosus* would be on the basis of a more acutely pointed tail in *S. suis;* but as this character is very variable, the distinction between *S. suis* and *S. papillosus* on morphological grounds is uncertain.

Sandground's statement with reference to the variability of the shape of the tail in the two species might be interpreted as implying that the forms from pigs and sheep overlap with regard to this morphological feature. As will be shown later, such overlapping has not been observed by the writers. On the contrary, the shape of the tail is remarkably constant for the sheep form as well as for the form from pigs, the difference between the two being very distinct and striking.

Baylis (*1*) says:

> *S. suis* von Linstow, 1905, a form originally recorded by Lutz in 1885 from the pig, appears to be a very doubtful species, and is probably identical with *S. papillosus*.

There are several additional references to Strongyloides in swine which, in the main, are repetitions of opinions expressed by other workers and supported by little, if any, original observation or critical examination of published data.

It is evident from this summary of the literature with reference to Strongyloides in swine that the views expressed by Grassi with reference to the specific identity of the sheep and swine forms have largely prevailed and that a number of later workers who have accepted these views have not supported them with morphological evidence based on original observations. A conspicuous exception is Ransom (*13*), who was familiar with the forms from the two hosts and did not consider them identical. It should also be noted that Hall (*5*) observed the discrepancies between descriptions by European investigators of the morphology of Strongyloides from swine and the forms from swine occurring in the eastern part of the United States

with which he was familiar, and that he also recognized the fact that *S. longus* is a synonym of *S. suis* only so far as the former has reference to forms from pigs.

OCCURRENCE OF STRONGYLOIDES IN AMERICAN SWINE

Aside from the brief references made by Ransom (*13*), Hall (*5*), and Sandground (*15*) to the occurrence of Strongyloides in American swine, there appear to be no published data concerning these parasites of domestic swine in the United States. In view of the recorded pathogenicity of Strongyloides, especially to young pigs, based on a study of clinical symptoms of infested animals by Perroncito (*10*), Reisinger (*14*), and Marotel (*8*), it appears important to determine whether a presumably important pathogenic parasite of young pigs has been overlooked by most American parasitologists. As a preliminary step to an experimental study of the pathogenicity of Strongyloides for pigs, it appeared essential to determine: (1) Whether one or more than one species of Strongyloides has been recorded from these host animals; and (2) whether the view shared by certain helminthologists with reference to the probable identity of *S. papillosus* and *S. suis* is supported by morphological and other evidence.

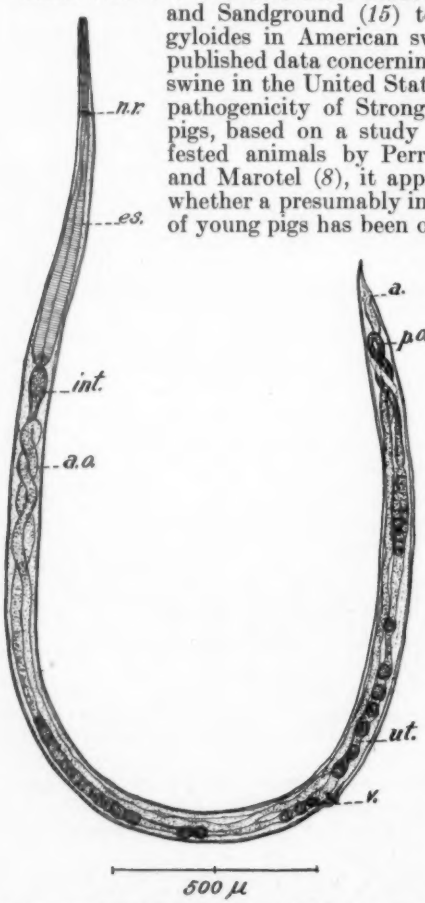


FIGURE 1.—*Strongyloides ransomi;* parasitic female. *a.*, anus; *a. o.*, anterior ovary; *int.*, intestine; *n. r.*, nerve ring; *es.*, esophagus; *p. o.*, posterior ovary; *ut.*, uterus; *v.*, vulva

Examinations of pigs at the Bureau of Animal Industry Experiment Station, Bethesda, Md., for the presence of Strongyloides were begun by the senior author in 1927. Cultures of feces from young pigs, from a few weeks to a few months old, revealed, after about 24 hours, the presence of rhabditiform larvae, and after 48 hours or longer the presence of filariform larvae and of free-living males and females, the number of males present being very few as compared with the number of females. In the course of post-mortem examinations of young pigs, which were killed at various times in connection with certain experiments, parasitic females belonging to the genus Strongyloides were obtained from the small intestine. An examination of these specimens showed that they differed in several important respects from *S.*

papillosus of sheep and also from the figures and descriptions of *S. suis* published by investigators in Europe.

In the course of necropsies on three young pigs at Moultrie, Ga., by E. B. Cram and E. W. Nighbert, of the Zoological Division, several slender nematodes were collected from the small intestine and forwarded to the senior author for determination. Unfortunately these worms were poorly preserved, so that when they were examined no morphological details could be made out, but the shape of the tail resembled that in figures of Strongyloides from pigs published by European workers. The female tail which is figured and described by Reisinger and figured in Fiebiger's textbook on parasitology is highly attenuated, in contrast to the conical tail with a more or less blunt tip found in all specimens of Strongyloides collected from swine at Bethesda. A recent restudy of the latter forms by the writers and a comparison of these forms with those from sheep showed that the swine form represents a species distinct from the sheep form and also distinct from the pig form as figured and described from Europe. The name S. *ransomi* is proposed for the American species, the name *S. suis* being retained for the time being for the forms with acutely pointed tails. The latter species probably occurs in this country, as forms resembling it were collected from swine at Moultrie, Ga.

Strongyloides ransomi, new species.

Specific diagnosis: Strongyloides:

Parasitic females (fig. 1). From 3.33 to 4.49 mm. long and from 54μ to 62μ wide. Body long, filiform, and of nearly equal thickness from the region of the base of the esophagus to the region of the posterior ovarian loops. Beginning at about the region of the base of the esophagus, the body narrows gradually toward the anterior end, the head being attenuated to a diameter of about 15μ. Posteriorly the body diminishes in size backward. The diameter of the body in the region of the anus is from 23μ to 31μ. In the region of the posterior ovarian loops the body narrows considerably and becomes gradually reduced behind the anus, terminating in a distinctly tapering, conical tail, the tip of which is more or less blunt. The esophagus is from 605μ to 883μ long by 47μ wide. The diameter of the body in the region of the base of the esophagus is from 47μ to 54μ wide. The anus is located at a distance of from 53μ to 83 μ from the posterior end. The vulva is a transverse slit, with salient lips, situated posterior to the middle of the body but considerably anterior to the last third of the body, at a distance of from 1.1 to 1.6 mm. from the tip of the tail. The ovary is twisted in loops anteriorly and is less constantly looped posteriorly. The posterior ovary is not uncommonly bent in a hairpin fashion, with a tendency to cross over and form a loop. (Fig. 2.) The eggs (fig. 3) are ellipsoidal, thin shelled, from 45μ to 55μ long, 26μ to 35μ wide, and contain an embryo at the time that they are eliminated with the feces.

Table 1 gives a summary of the measurements of eight specimens of parasitic females.

TABLE 1.—*Measurements of eight specimens of parasitic females of Strongyloides ransomi*

Size		Esophagus		Location of vulva		Length of tail
Length	Width	Length	Width	From anterior end	From posterior end	
Mm.	μ	μ	μ	*Mm.*	*Mm.*	μ
4.495	62	853	47	2.821	1.674	79
3.72	54	760	47	2.325	1.395	67
4.03	62	605	47	2.968	1.162	68
4.65	62	853	47	2.99	1.66	83
3.332	62	713	47	1.922	1.41	71
3.952	62	822	47	2.821	1.131	67
3.689	62	883	47	2.124	1.565	53
3.67	62	775	47	2.245	1.425	79

Free-living generation:

Males (figs. 4 and 5). From 868μ to 899μ long and 54μ wide. Body of nearly equal diameter except in the tail region. Posterior to the anus the body becomes considerably narrowed, tapering gradually and terminating in a relatively long, slender tail; anteriorly the body narrows gradually, commencing in the region of the base of the esophagus. The mouth leads into a short pharynx; the esophagus is from 132μ to 140μ long and 23μ wide in the posterior bulbous portion. Spicules from 26μ to 29μ long, shaped like curved blades and having a knob-like handle. The gubernaculum is from 18μ to 18.7μ in maximum diameter, and 9.4μ in minimum diameter. One preanal and one postanal papilla, ventral in position, approximately equidistant from the anus. The tail is from 83μ to 90μ long.

FIGURE 2.—*Strongyloides ransomi;* posterior end of parasitic female

FIGURE 3.—Eggs of *Strongyloides ransomi*

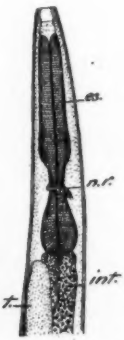


FIGURE 4.—*Strongyloides ransomi;* anterior end of male. *int.*, intestine; *n. r.*, nerve ring; *es.*, esophagus; *t.*, testis

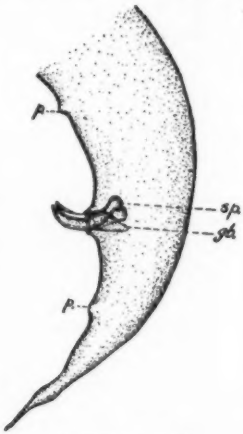


FIGURE 5.—*Strongyloides ransomi;* posterior end of male. *gb.*, gubernaculum; *p.*, papilla; *sp.*, spicule

Females (fig. 6). From 1 to 1.1 mm. long and 62μ wide in the region of the vulva. The body tapers very gradually anteriorly and narrows to a diameter of about 13μ to 15μ in the head region; the diameter of the body in the region of the esophagus is from 43μ to 47μ. Posteriorly the body tapers more abruptly than anteriorly, becoming considerably narrowed in the region of the rectum and tapering gradually to a slender tail. The diameter of the body in the region of the anus is about 23μ. The mouth leads into a short pharynx; the esophagus is from 124μ to 155μ long and 23μ wide in the posterior bulb. The vulva has salient lips and is located near the middle of the body. In young forms comparatively few ovarian eggs are present in the uterus, but in gravid females numerous shelled eggs are contained in the anterior and posterior uteri. After oviposition is completed the uterus shrinks in size and the ovaries appear to be degenerated. The tail is from 150μ to 158μ long.

Rhabditiform larvae:

The rhabditiform larvae (figs. 7 and 8) are from about 280μ to somewhat over 400μ long and about 20μ in maximum width, the smaller forms being newly hatched. The pharynx is short, from 5μ to 6μ long. The esophagus is from about 70μ to nearly 90μ long. The genital primordium, which is about 15μ to 18μ long, is located approximately in the region corresponding to that of the middle of the intestine. The tail is tapering, about 55μ long.

Filariform larvae:

These larvae (fig. 9) are from 504μ to 635μ long and from 15μ to 19μ wide in the region of the base of the esophagus. The esophagus is from 240μ to 310μ

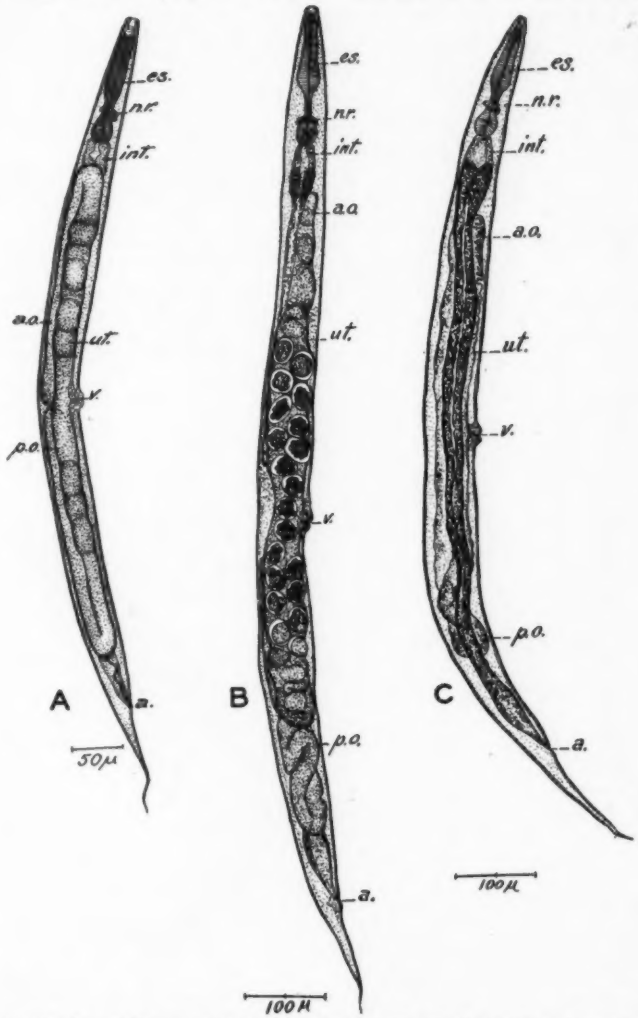


FIGURE 6.—*Strongyloides ransomi:* A, Young female; B, gravid female; C, female after oviposition. *a.*, anus; *a. o.*, anterior ovary; *int.*, intestine; *n. r.*, nerve ring; *es.*, esophagus; *p. o.*, posterior ovary; *ut.*, uterus; *v.*, vulva

long and from 7.5μ to 11μ wide. The tail is tapering from 60μ to 90μ long. The genital primordium is located at a distance of from 168μ to 225μ from the tip of the tail, approximately in the region corresponding to that of the middle of the intestine.

Host.—Sus scrofa domestica.

Location.—Small intestine (embedded in mucosa). Free-living generation and rhabditiform and filariform larvae may be cultured in feces of infested hosts.

Type locality.—Bethesda, Md.

Type specimen of parasitic female.—Bureau of Animal Industry helminthological collection, United States National Museum No. 28779.

Paratypes of parasitic female.—Bureau of Animal Industry helminthological collection, United States National Museum No. 28780.

Type specimens of free-living male and female.—Bureau of Animal Industry helminthological collection, United States National Museum No. 28777.

Paratypes of free-living male and female.—Bureau of Animal Industry helminthological collection, United States National Museum No. 28778.

This species is dedicated to the memory of the late Brayton Howard Ransom, who reported, for the first time, Strongyloides from swine in the United States and who carried out important experimental work on skin penetration by *S. papillosus* and on the experimental transmission of this species to rabbits.

FIGURE 7.—Newly hatched rhabditiform larva of *Strongyloides ransomi*. *a.*, anus; *g. p.*, genital primordium; *int.*, intestine; *n. r.*, nerve ring; *es.*, esophagus

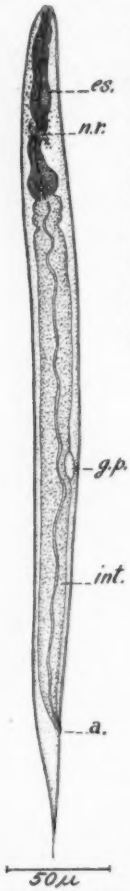


FIGURE 8.—Fully grown rhabditiform larva of *Strongyloides ransomi*. *a.*, anus; *g. p.*, genital primordium; *int.*, intestine; *n. r.*, nerve ring; *es.*, esophagus

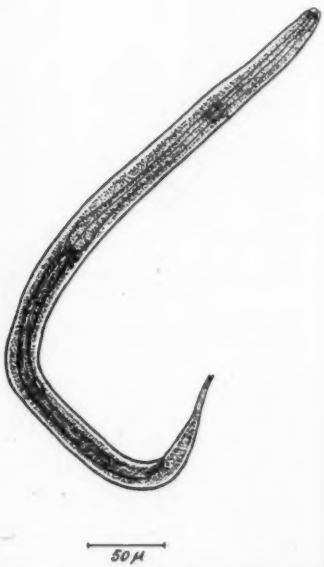


FIGURE 9.—Filariform larva of *Strongyloides ransomi*

COMPARISON OF STRONGYLOIDES RANSOMI WITH S. PAPILLOSUS

In a comparison of the parasitic females of *Strongyloides ransomi* with *S. papillosus*, the shape of the tail presents a striking difference. That of *S. papillosus* (fig. 10) is of fingerlike shape and markedly

blunt, whereas that of *S. ransomi* (fig. 11) is cone-shaped and tapers gradually, its tip being more or less bluntly rounded. Among numerous specimens of parasitic females from sheep and swine which were examined, the shape of the tail was found to be constant for each species, and the variations were not found to overlap. Aside from

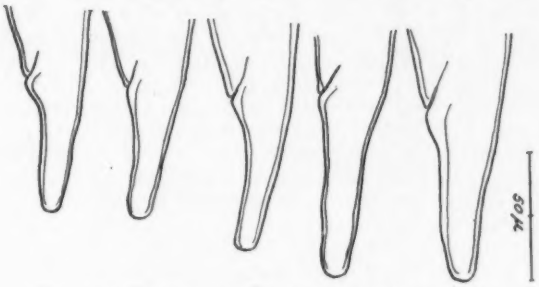


FIGURE 10.—Tails of parasitic females of *Strongyloides papillosus*

this conspicuous difference, no other highly significant morphological differences have been found in comparing the forms from the two hosts. As regards the size of the eggs, those of *S. ransomi*, obtained from fresh pig feces, showed a range of from 45μ to 55μ long by 26μ

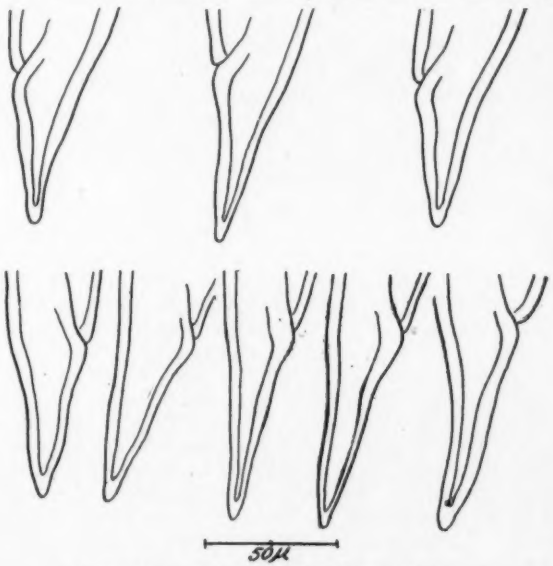


FIGURE 11.—Tails of parasitic females of *Strongyloides ransomi*

to 35μ wide, whereas those obtained from fresh sheep feces showed a range of from 48μ to 67.5μ long by from 30μ to 34μ wide. It is of interest to note that of 10 measured eggs of *S. ransomi* 8 were less than 50μ long and 2 were between 50μ and 55μ long, whereas 12 of 20 eggs of *S. papillosus* ranged from 60μ to 67.5μ in length, and only 1

egg was less than 50μ long, the remaining 7 eggs being between 52μ and 56μ long. In a general way the eggs of *S. papillosus* are longer and more elongated than those of *S. ransomi*.

It is also noteworthy that whereas cultures of feces from swine infested with *Strongyloides ransomi* have yielded in all cases free-living males, cultures of feces from sheep infested with *S. papillosus* have thus far failed to show the presence of males. Ransom (*12*) carried out a number of experiments on the transmission of *S. papillosus* from sheep to rabbits, and he apparently failed to find males in his cultures. In 1911 he gives a fairly detailed description of the free-living female and states that the male is undescribed. Baylis (*1*) states that the male of *S. papillosus* is apparently rare. He gives the first description of the free-living male of *S. papillosus* based on one specimen as follows: 750μ long by 35μ wide, tail 70μ long, spicules 35μ long. While the size measurements are consistently smaller than those recorded in this paper for *S. ransomi*, it is significant that the spicules of *S. papillosus* are considerably longer than those of *S. ransomi*, according to Baylis's measurements of those of the former species. The spicules of four specimens of *S. ransomi* showed the following lengths: 26μ, 28μ, 28μ, 29μ. It is to be regretted that Baylis does not give the host from the feces of which the male was cultured. One can not assume with absolute certainty that the host in question was a sheep, since Baylis lists the following hosts for *S. papillosus*: Sheep, goat, ox, pig, and rabbit.

As regards the filariform larvae, 6 specimens of *Strongyloides ransomi* showed the following range in size: 503μ long, 16μ wide; 635μ long, 18μ wide; 519μ long, 18μ wide; 542μ long, 18μ wide; 519μ long, 16μ wide; 496μ long, 15μ wide. Seven filariform larvae of *S. papillosus* showed the following range in size: 651μ long, 17μ wide; 666μ long, 15μ wide; 681μ long, 15μ wide; 710μ long, 15μ wide; 651μ long, 15μ wide; 574μ long, 15μ wide; 666μ long, 15μ wide. While these measurements overlap, it is important to note that the filariform larvae of *S. papillosus* are considerably longer than those of *S. ransomi*, the maximum size of the latter being close to the minimum size of the former. The size of the esophagus of the filariform larvae in the two species shows a variation of from 230μ to 279μ in length by from 11μ to 15μ in width for *S. papillosus*, and a variation of from 240μ to 310μ in length by from 7.5μ to 11μ in width for *S. ransomi*. While these measurements overlap, it is important to note that the individual measurements of the esophagus for the two species show differences as follows: *S. papillosus:* 240μ by 11μ, 271μ by 11μ, 263μ by 11μ, 279μ by 15μ, 255μ by 11μ, 230μ by 11μ, and 279μ by 11μ; *S. ransomi:* 255μ by 7.5μ, 310μ by 7.5μ, 240μ by 7.5μ, 263μ by 11μ, 263μ by 7.5μ, and 255μ by 7.5μ. Aside from the fact that the base of the esophagus of the filariform larvae of *S. papillosus* is wider in most specimens than that of *S. ransomi*, the ratio of the length of the esophagus as compared to the total length of the body is considerably larger in the latter species. The location of the genital primordium with reference to its distance from the anus in the two species is as follows: *S. ransomi:* 121μ, 125μ, 109μ, 118μ, 105μ; *S. papillosus:* 147μ, 152μ, 170μ, 170μ, 170μ, 151μ, 165μ.

Aside from the morphological differences between *S. ransomi* and *S. papillosus* which have been noted, there also appears to be a biological difference between these two species, so far as concerns their ability to develop in the rabbit. As first shown by Ransom (*12*),

S. papillosus can develop to fertile maturity in rabbits. In order to determine whether there are any differences between *S. ransomi* and *S. papillosus* with regard to the degree of adaptability to various abnormal hosts, the following experiments were performed:

Filariform larvae of *S. ransomi* were fed to a cat, a guinea pig, a rat, two rabbits, and a chick. These animals were held under observation for varying periods ranging from about three weeks to two months, during which interval the feces were examined for eggs and were cultured for the presence of larvae, with negative results in all cases. One rabbit in this series was fed filariform larvae on April 3, 1929; the animal died on April 24 and a careful examination failed to reveal the presence of Strongyloides in the intestine. The second rabbit was fed filariform larvae on May 7, 1929, and was killed on July 6; during this interval no eggs were detected in the feces and on post-mortem examination no Strongyloides were found in the intestine.

Filariform larvae of *S. papillosus* were fed to two rabbits, two guinea pigs, and a chick. One rabbit, which was fed larvae on April 13 and 15, failed to show eggs in the feces up to May 10, on which day it was chloroformed. At necropsy 54 specimens of Strongyloides, most of which were not gravid, were collected from the intestine. The second rabbit was fed filariform larvae of *S. papillosus* on May 21, 1929; eggs were first noted in the feces of this rabbit on June 17 and continued to be eliminated with the feces up to July 8, during which interval several fecal examinations were made. Since July 11 the feces of this rabbit have been consistently negative for eggs, and cultures of the feces have failed to show the presence of larvae. The guinea pigs and chick were kept under observation more than six weeks, during which interval they showed no eggs in the feces; these animals showed no Strongyloides at necropsy.

It is evident from these experiments that *S. papillosus* can develop to fertile maturity in rabbits, while *S. ransomi* is apparently not adapted to this host.

FIGURE 12.—*Strongyloides suis.* (From Reisinger, 1915)

STRONGYLOIDES SUIS

The status of the name *Strongyloides suis* still remains to be considered. As used by Von Linstow (*6*), this name has little, if any, zoological standing, as it is not accompanied by a description. Von Linstow apparently had in mind the species of Strongyloides from swine described by Lutz (*7*) which is described, however, without reference to specific details, the characters mentioned in the description being, with the exception of the size of the females, generic. For the time being, the name *S. suis* may be retained for the form described and figured by Reisinger (*14*) which, so far as can be judged from his illustration, appears to be identical with that figured by Fiebiger (*2*) from swine. Reisinger's species may be characterized as follows:

Parasitic females (fig. 12). From 4 to 6 mm. long by 80μ wide, attenuated at the anterior end. Esophagus one-fifth of the total length. Uterus contains

20 to 30 eggs. Vulva near the posterior fourth of the body. Eggs 60μ long by 40μ wide containing an embryo three and one-half to four times as long as the egg. Tail long and acutely pointed.

Reisinger failed to obtain the free-living generation in cultures. The forms which he figures as possibly representing the free-living males and females were obtained from feces in hogpens and are unquestionably free-living nematodes.

The striking character of Reisinger's form is the acutely pointed tail, which so far as is known, and if accurately figured, is unique in the genus Strongyloides. The position of the vulva, approximately in the beginning of the posterior fourth of the body, represents another difference between this form and *S. ransomi*, in which latter the vulva in the parasitic female is located at some distance anterior to the beginning of the posterior third of the body.

In view of the fact that Fiebiger's figure of Strongyloides from pigs, designated by him as *S. longus*, corresponds to that of Reisinger, at least so far as the shape of the female tail is concerned, and that forms resembling these worms were collected from pigs in Georgia, in which case it is to be noted that symptoms of digestive disturbance were observed and ascribed to these parasites, the recognition of these forms as a species distinct from *S. ransomi* is warranted. For the time being the name *S. suis* which has been used by many helminthologists for the species from swine may be retained for the long-tailed European forms, largely as a matter of convenience. The retention of this name is also justified from the standpoint of nomenclature.

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SOME EFFECTS OF SEED TREATMENT ON THE GERMINATION AND SUBSEQUENT GROWTH OF WHEAT [1]

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INTRODUCTION

Where winter wheats can be successfully grown they outyield spring wheats. For the years 1910 to 1927, inclusive, the difference in yield for the country as a whole was 15.7 per cent in favor of winter wheat (*17*).[2] When winterkilling occurs, it is sometimes possible to reseed with winter wheat early in the spring or during the late winter. The question as to how late winter wheat may be spring-sown and yet mature normally is of vital interest to the wheat grower under such conditions.

It is a well-recognized fact that true winter wheats sown at spring planting time will not head until late fall or the following summer. This condition has been termed "vegetative dormancy," which, although similar, is hardly analogous to the dormancy of seeds of woody plants.

A recent case of vegetative dormancy was noted in Peru. "The winter wheats, Hybrid 128, Fortyfold, and Turkey failed to produce culms when planted in early June, which is the beginning of the fall season. They reacted much like winter wheats planted in the spring in the States. Spring varieties produced a normal growth." [3]

Several causes have been assigned for the failure of winter wheat to mature normally when spring seeded. The growth habit is a heritable character. Aside from this, there are certain environmental factors which seem necessary for the expression of the character of winter habit. Exposure to freezing in the field, favorable light relations, and nutritional factors have been suggested as indispensable to normal growth. Investigators have shown that these factors do affect the growth periods.

REVIEW OF LITERATURE

Many workers have studied the effects of seed treatment on the germination of seeds. Comparatively few, however, have studied the growth of plants produced from treated seeds past the very earliest growth stages. The literature here cited is chiefly that which deals with subsequent growth.

Tincker (*16*) found that soaking oat grains in water accelerated seedling growth and concluded that a definite correlation existed between the vigor of germination and the rate of subsequent growth. Jones and Tincker (*9*) state that seed stimulation as an aid to rapid growth of seedlings was found to affect yields of oats.

Kiessling (*11*), using a large number of chemicals, was able to increase the germination of wheat, oats, and barley. Gleisberg (*6*)

[1] Received for publication Apr. 16, 1929; issued January, 1930.
[2] Reference is made by number (italic) to "Literature cited," p. 35.
[3] E. V. Abbott, Plant Pathologist, Estacion Experimental Agricola, Lima, Peru. Correspondence.

increased the vegetative growth of radish 364 per cent by treating the seeds with a solution containing 15 per cent magnesium chloride and 15 per cent magnesium sulphate. Seed treated in distilled water yielded more than twice that of the control. Silbert (*15*) reported increased yields and somewhat earlier maturity from Canada field peas, soybeans and buckwheat when treated with a solution of copper sulphate. Popoff (*14*) concluded that it was possible to increase the yields of crops 30 to 50 per cent by treating the seeds with solutions of chemicals before planting.

Denny (*3*) studied the effects of numerous organic chemicals on freshly harvested potatoes. With many of these he was able to overcome the dormant period and force sprouting.

Several investigators have studied the effects of different temperatures on seeds. Munerati (*13*) concluded that fresh seed exposed to low temperatures at the germination period made a higher and a more rapid germination. Fawcett (*4*) found that exposure of weed seeds to low temperatures with intermittent thawing increased the germination and shortened the dormant period. Coffman (*2*) states that small grains will germinate at the temperature of melting ice.

Jensen (*8*) noted little or no effect on subsequent vegetative growth after soaking wheat in water and freezing it before planting. Howard (*7*), working with 65 species of woody plants, found that freezing was the best treatment for forcing growth. Kidd and West (*10*) have made a comprehensive review of the literature on physiological predetermination. Of the work mentioned, that of Gassner is of the most interest. He found that exposure of the seeds of several winter cereals to low temperatures during germination or early seedling growth assures flowering the first year. The earlier in the development of the plant the treatment was given the more pronounced the results obtained. Similar treatment with summer annuals did not appreciably affect the time at which culm formation took place.

The effects of light on the vegetative periods of plants have been studied by several investigators. Garner and Allard (*5*) state that in any region the relative lengths of day and night constitute one of the controlling factors in plant development. Klages (*12*) concluded that there was no material difference between winter and spring wheat growth habits when the plants were grown in the greenhouse. Wanser (*18*) concluded that the "photoperiod" or favorable length of day was the key to the distinction between winter and spring wheats.

MATERIALS AND METHODS

The purpose of the investigation herein reported was to determine the possibility of using chemical or freezing treatments of germinating wheat seed as a means of breaking or modifying the vegetative dormancy of the resulting plants.

The work as carried out required nine hundred and sixty-nine 8-foot rows in the field trials. Germination counts were made on 340 lots of treated wheat. Root and sprout lengths in millimeters were taken on 3,400 seedlings grown in a standard germinator. Germination and seedling vigor were used as indices of the effects of seed treatments and formed the bases for determining the most desirable concentrations of solutions for field trials. Both germination and field trials were run in duplicate.

The following 16 varieties of wheat were used: White Winter, Triplet, Turkey, Blackhull, Kharkof, Ridit, Fortyfold, Hybrid 128, Oro, White Odessa, Kanred, Hybrid 63, Hybrid 143, Hard Federation, Marquis, and Baart. Pure seed from nursery breeding trials was used in this experiment. It is realized that hand-threshed pure-line seed of each variety would have been better. Seed of this type, however, was not available. All heads formed were carefully checked

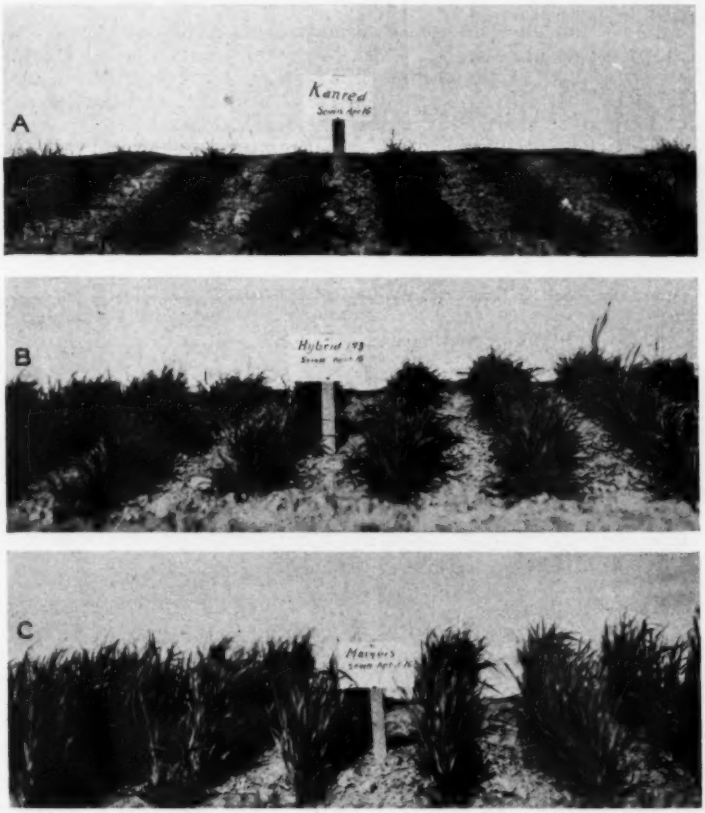

FIGURE 1.—Early growth habits of typical winter, semiwinter, and spring wheats, sown April 16 and photographed 34 days after spring seeding: A, Kanred winter wheat; B, Hybrid 143 semiwinter wheat; C, Marquis spring wheat

for varietal characteristics and were field sown in the spring of 1928 to determine the habits of growth.

The first 11 varieties are of the winter type. Hybrids 143 and 63 are semiwinter and the last three are spring wheats. Figure 1 shows the early growth habits of these three types of wheat. Ridit is a hard red winter selection from the cross Turkey × Florence, made at Pullman, Wash. Oro is a Turkey selection made at Moro, Oreg. The other varieties are described in United States Department of Agriculture Bulletin 1074 (*1*).

The chemicals, the length of treatment, and the concentrations used are listed below.

Trichloroethylene, 3 hours in a 6 per cent solution.
Ethylene chloride, 3 hours in a 1 per cent solution.
Ethylene chlorhydrin, 3 hours in an 8 per cent solution.
Ethyl bromide, 3 hours in a 6 per cent solution.
Ammonium thiocyanate, 3 hours in a 3 per cent solution.
Potassium thiocyanate, 3 hours in a 1 per cent solution.
Carbon bisulphide, 2 hours in water and 1 hour in a 3 per cent solution.

These chemicals were used by Denny (*3*) in hastening the sprouting of dormant potato tubers. With the exception of the thiocyanates, they are liquids in the commercial form. It is recognized that trichloroethylene, ethylene chloride, ethyl bromide, and carbon disulphide are insoluble or only slightly soluble, so true solutions are not formed with water in some cases. The mixture of chemical and water, however, will be referred to as a solution.

The solutions were made and the treatments given in earthen bowls at a temperature of approximately 20° C. The grain was weighed in 10-gm. lots and placed in cheesecloth bags for treating. To avoid differences in concentration of solutions all varieties were treated

FIGURE 2.—General view of the field nursery in which wheats were grown after seed treatment with various chemicals; the winter wheats are in the foreground, semiwinter at the lower center, and the spring wheats immediately behind the semiwinter

simultaneously in the same solutions. After submersion, the bags were allowed to drain one and one-half hours, after which the seed was field planted or transferred to germination blotters.

The lots frozen were first soaked in water 1 hour and allowed to remain in the germinator 20 hours before removal to the cold chamber. Moisture tests showed that the moisture content was about 18 per cent. This procedure allowed the seeds to begin germination before being frozen. Freezing was done in a dairy refrigeration room at a constant temperature of −12° C. Freezing was followed by chemical treatment. The treated material for all lots was planted in the field April 16, 1927, in 8-foot rows at the rate of 2 bushels per acre and at a depth of 2 inches.

Four series of trials were run in duplicate. New and aged seeds were used. Freezing was used alone and in combination with chemical treatment. Both wet and dry checks were included. Germination and seedling-growth studies were made as an index of the effect of seed treatment for determining the concentrations of solutions to be used. Root growth, germination, and sprout growth were recorded. For the field-seeded material the percentages of survival and the growth habits were determined. Dates of first heading, full heading, and ripening were observed. The numbers of heads of winter wheat which ripened by September 1 were recorded.

DISCUSSION AND RESULTS

EFFECT OF TREATMENTS ON NUMBER OF HEADS PRODUCED

Table 1 shows the number of heads produced by winter wheats in the first series of treatments when the chemicals as previously listed were used. Figure 2 shows a general view of the field nursery. The relative growth of the three types of wheat is clearly shown. In this series the grain was soaked one hour in running water at a temperature of 17° C. This soaking gave a moisture content of approximately 18 per cent. The grain was drained 10 minutes and placed in the solutions for treatment. After treatment the lots were immediately seeded in the field. Two checks were used, one a dry, untreated lot and the other soaked in water for a period corresponding to the length of the chemical treatments.

TABLE 1.—*Number of heads produced by spring-sown winter wheats after seed treatment with various chemicals*

[Duplicate rows 8 feet in length]

SERIES 1

Chemical	Number of heads produced by wheat varieties indicated								Total
	Turkey	Blackhull	Kharkof	Fortyfold	Hybrid 128	Oro	Ridit	White Odessa	
Potassium thiocyanate	14	5	4	2	0	5	0	0	30
Ammonium thiocyanate	0	0	7	0	0	1	0	0	8
Trichloroethylene	2	6	7	2	0	3	0	0	20
Ethylene chlorhydrin	2	6	1	3	0	2	0	0	14
Ethylene chloride	1	11	4	1	0	0	0	0	17
Ethyl bromide	1	6	10	0	0	4	0	0	21
Carbon disulphide	3	3	4	0	0	1	0	0	11
Wet check	4	6	4	1	0	0	0	0	15
Dry check	0	0	7	2	0	2	0	0	11
Total	27	43	48	11	0	18	0	0	147

SERIES 2

Potassium thiocyanate	1	9	5	0	0	1	0	0	16
Ammonium thiocyanate	0	5	1	0	0	0	0	0	6
Trichloroethylene	9	10	6	0	0	0	0	0	25
Ethylene chlorhydrin	5	7	11	1	0	2	0	0	26
Ethylene chloride	4	11	9	1	0	3	0	0	28
Ethyl bromide	1	24	5	1	0	3	0	0	34
Carbon disulphide	5	10	6	2	0	1	0	0	24
Wet check	4	9	9	2	0	1	0	0	25
Dry check	3	7	9	4	0	0	0	0	23
Total	32	92	61	11	0	11	0	0	207

The heads of winter wheat which appeared in 1927 were harvested, and as a check for mixtures they were field-seeded in the spring of 1928. The varieties Kanred, White Winter, and Triplet contained true spring-wheat strains which had the typical morphological characters of the parent varieties. Because of the presence of these spring strains the results for the above varieties are not given in Table 1. The spring types will be grown in the nursery in 1929 for further study.

It may be noted (Table 1) that potassium thiocyanate produced more heads than other treatments, ethyl bromide was second and trichloroethylene third. Ammonium thiocyanate produced the fewest. The variety Kharkof produced the most heads, and Blackhull slightly fewer. Hybrid 128, Ridit, and White Odessa failed to produce heads. True spring and semiwinter wheats were fully headed at the normal dates.

In Table 1 data are also given for series 2. This series is a duplication of series 1 except that before being planted the treated grain was placed in a standard germinator for 20 hours at a temperature of 21° C. Of the chemicals used, ethyl bromide produced the largest number of heads, with ethylene chloride closely following. With the exception of ammonium thiocyanate, potassium thiocyanate, and carbon disulphide, the chemical treatments produced an increase in the number of heads. These increases are not large enough to justify definite conclusions.

As in series 1 so in series 2 the grain treated with ammonium thiocyanate produced fewest heads. It may be noted that the treatments, based on number of heads produced, do not rank as in the first series. With the exception of the thiocyanate treatments, each produced more heads in series 2 than in series 1. Taking the total number of heads for each series, it will be seen that series 2 produced 40 per cent more heads than series 1. More heads were produced by all varieties heading in series 2 than in series 1 with the exception of Fortyfold which produced the same number in both series and Oro which produced a greater number of heads in series 1. The only difference between these series was the incubation of the treated grain used in the second series.

To get the general effect of the chemicals and the reaction of the varieties the figures for both series have been combined, although the treatments in each case are not the same. Combining the results of both series, the treatments rank as follows on a basis of heads produced: Ethyl bromide, 55; potassium thiocyanate, 46; trichloroethylene, 45; ethylene chloride, 45; wet check, 40; ethylene chlorhydrin 40; carbon disulphide, 35; dry check, 34; ammonium thiocyanate, 14.

Similarly, when heads produced by each variety are grouped for both series, the varieties rank as follows: Blackhull, 135; Kharkof, 109; Turkey, 59; Oro, 29; Fortyfold, 22; White Odessa, 0; Hybrid 128, 0; Ridit, 0.

Since the number of heads produced was the only variable one noted, this is used to represent the effects of the treatments. The heads produced by a variety are assumed to represent the relative degree to which that variety is vegetatively dormant. Ridit, White Odessa, and Hybrid 128 may be said to more dormant that Blackhull.

Freezing of seeds in a germinated condition, combined with chemical treatments, did not break the vegetative dormancy of the resulting plants. Table 2 gives a summary of data for White Winter and

Kanred, new and aged seed being used. The figures represent percentage of stand and heads produced. It should be remembered that from these two varieties spring strains were derived when ripened heads were used as seed the following year. In this series the thiocyanates show a decided reduction in stand and heads produced. Trichloroethylene-treated seeds show an increase in number of heads over the average of the checks, but not higher than the check frozen dry, which produced the best stand and the largest munber of heads.

TABLE 2.—*The effect upon stand and head production of soaking wheat seed in water 1 hour, allowing it to germinate 20 hours, freezing it 12 days at −12° C., and treating it with various chemicals*

[Duplicate rows 8 feet in length]

Treatment	Results with Kanred, using—				Results with White Winter, using—			
	Previous crop seed		1923 crop seed		Previous crop seed		1925 crop seed	
	Stand [1]	Heads produced [2]	Stand	Heads produced	Stand	Heads produced	Stand	Heads produced
	Per cent	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*
Potassium thiocyanate	50	1	16	1	75	2	40	0
Ammonium thiocyanate	30	1	20	0	75	0	10	0
Trichloroethylene	90	8	50	2	100	8	75	2
Ethylene chlorhydrin	75	4	75	0	100	9	75	0
Ethylene chloride	90	2	50	2	100	5	80	0
Ethyl bromide	75	7	85	0	90	5	56	0
Carbon disulphide	85	8	75	0	100	1	80	0
Check, frozen wet	90	8	50	0	100	3	50	0
Check, frozen dry	100	5	75	3	100	12	100	3
Unfrozen check	100	7	100	1	100	1	100	2
Average	78.5		59.6		94.0		66.6	
Total		51		9		46		7

[1] 100 per cent stand is 100 plants. [2] Heads produced by Sept. 1.

Freezing 12 days at −12° C. did not entirely kill seeds of either variety of wheat. Frozen dry, both varieties germinated normally or nearly so. Frozen wet, from 10 to 50 per cent of the germs were killed. Kanred, in other trials gave a lower germination than Marquis after 12 days of freezing of soaked seed. Allowing frozen grain to thaw for 12 hours at any time in the 12-day freezing period resulted in a complete killing of every variety frozen. This would indicate that the length of the freezing period was less important than the occurrence of intermittent thawing.

A marked reduction in vitality of seed is indicated by the lower percentage of stand and number of heads produced by aged seed. (Table 2.) The amount of the reduction of germination and heading was not directly proportional to the age of the seed. Kanred produced a higher percentage of heads per plant than did White Winter.

EFFECT OF TREATMENTS ON GERMINATION OF SEEDS AND ON GROWTH OF ROOTS AND SPROUTS

Tables 3 to 5, inclusive, show data on germination, root growth, and sprout growth of grain treated with chemicals and allowed to germinate in a standard germinator at a temperature of 21° C. Germination counts were made at 48 and 96 hour periods after incubation, and root and sprout growth were measured at 96 and 144 hour periods.

TABLE 3.—*The effect of chemical treatments of the seed on the germination of four varieties of wheat*

[Hours after incubation in a standard germinator at 21° C.]

Treatment	Percentage germination of—							
	Kanred after—		White Winter after—		Marquis after—		Hybrid 143 after—	
	48 hours	96 hours	48 hours	96 hours	48 hours	96 hours	48 hours	96 hours
Potassium thiocyanate	95	100	92	100	100	100	93	100
Ammonium thiocyanate	51	62	37	38	72	87	58	59
Trichloroethylene	98	100	91	100	100	100	100	100
Ethyl bromide	93	100	19	56	97	100	97	100
Ethylene chloride	98	100	92	100	97	100	99	100
Carbon disulphide	97	100	89	100	99	100	96	100
Average	88.7	93.7	70.0	82.5	94.2	97.8	90.5	93.2
Check (no treatment)	97	100	93	100	100	100	96	100

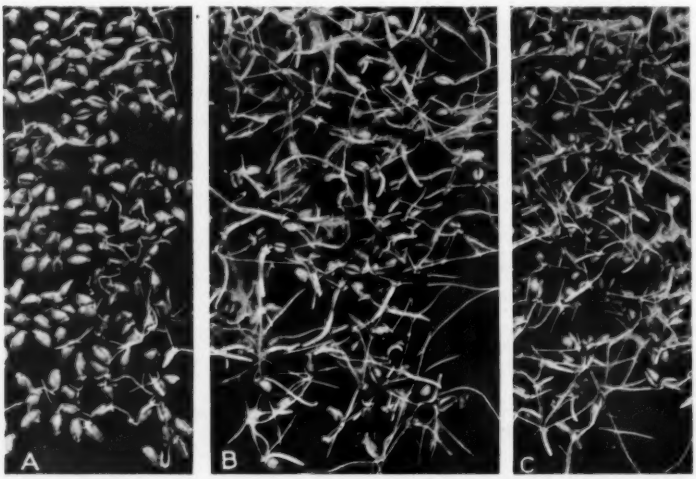

FIGURE 3.—Growth of winter-wheat seedlings in a standard germinator at a temperature of 21° C. 72 hours after treatment with ammonium thiocyanate (A), and trichloroethylene (C), compared with the untreated control (B)

Table 3 shows that the germination of the four varieties studied was rather uniform except for the marked reduction in germination of White Winter by ethyl bromide. Ammonium thiocyanate markedly reduced germination in all varieties.

Root growth (Table 4) was more variable for varieties and treatments than germination or sprout growth. Treatments of Marquis and Hybrid 133 increased root growth, but in general this was not true for Kanred and White Winter. Treatment with ammonium thiocyanate reduced root growth considerably below that of the check in Kanred and White Winter.

The data for sprout growth are given in Table 5. Ammonium thiocyanate reduced sprout growth of all varieties. No sprouts from treated seed were as vigorous as the checks. Kanred showed better sprout vigor than other varieties.

The variations in the effects of different chemicals are small except those of ammonium thiocyanate and ethyl bromide. Figure 3 shows the effects of ammonium thiocyanate and trichloroethylene on seedlings of White Winter wheat.

TABLE 4.—*The effect of chemical treatments of the seed on the root growth of four varieties of wheat*

[Hours after incubation in a standard germinator at 21° C.]

Treatment	Length in millimeters of roots of—							
	Kanred after—		White Winter after—		Marquis after—		Hybrid 143 after—	
	96 hours	144 hours	96 hours	144 hours	96 hours	144 hours	96 hours	144 hours
Potassium thiocyanate	46	52	37	52	44	64	35	51
Ammonium thiocyanate	32	37	17	25	39	51	28	39
Trichloroethylene	41	56	33	46	41	59	45	53
Ethyl bromide	41	50	26	46	37	50	47	56
Ethylene chloride	48	54	34	50	46	63	46	52
Carbon disulphide	40	54	35	53	43	58	44	59
Check (no treatment)	43	56	41	56	15	37	15	34
Average	41.3	50.5	30.3	45.3	41.7	57.5	40.8	51.7

TABLE 5.—*The effect of chemical treatments of the seed on the sprout growth of four varieties of wheat*

[Hours after incubation in a standard germinator at 21° C.]

Treatment	Length in millimeters of sprouts of—							
	Kanred after—		White Winter after—		Marquis after—		Hybrid 143 after—	
	96 hours	144 hours	96 hours	144 hours	96 hours	144 hours	96 hours	144 hours
Potassium thiocyanate	21	37	10	19	17	37	9	22
Ammonium thiocyanate	11	24	4	10	9	22	5	11
Trichloroethylene	23	45	8	24	14	41	11	25
Ethyl bromide	23	43	7	22	13	34	13	30
Ethylene chloride	20	41	9	28	20	45	12	31
Carbon disulphide	22	40	11	30	12	47	12	25
Check (no treatment)	22	43	14	31	21	45	15	35
Average	20	38.3	8.2	22.2	14.2	37.7	10.3	24

RELATION OF TIME OF PLANTING TO HEADING

Date of seeding is an important factor in determining the vegetative growth curve of winter wheats. An individual reaction of varieties to time of planting is noted when heading and subsequent maturity are considered.

To obtain the relation of the date of spring planting to heading, five varieties of wheat were sown at weekly intervals from January to May in 1926, 1927, and 1928. Two of the varieties, Marquis and Federation, are of spring-growth habit, and three varieties, Kharkof, Hybrid 128, and White Winter, are of winter habit. The latter variety was not seeded in 1926.

In each season the early seedings gave normal growth in all varieties. Later seedings, however, gave varied results except with Marquis, which headed normally for every date of seeding. The last dates of seeding used in these trials are given for the variety Marquis in Table 6.

TABLE 6.—*Latest date of seeding for normal heading of various varieties of wheat*

[Rows 8 feet in length]

Variety	Habit of growth	Latest date of sowing which permitted normal heading in—		
		1926	1927	1928
Marquis	Spring	[1] May 16	[1] May 23	[1] May 26
Federation	do	Apr. 26	May 23	May 12
Kharkof	Winter	Jan. 31	Mar. 5	Mar. 17
Hybrid 128	do	Jan. 31	Feb. 26	Mar. 10
White Winter	do	----------	Mar. 19	Mar. 17

[1] Last date sown.

These results show that the heading of Hybrid 128 is greatly influenced by time of spring seeding. In the order of the effect of time of planting on heading, the varieities may be listed as follows: Hybrid 128, Kharkof, White Winter, Federation, and Marquis. The effect of season on the heading of spring-planted winter wheats was shown. The 1927 and 1928 seasons were approximately a month later than the 1926 season. Kharkof headed when planted 33 days later in 1927 and 46 days later in 1928 than for the last date of planting for heading in 1926. Four-year trials at Moro, Oreg., gave similar results.

SUMMARY

The effects of various seed treatments on the germination of wheat seed and on the growth of the plants subsequently produced has been studied. Briefly, none of the treatments used proved to be either highly stimulative to growth or a modifier of growth habits of winter, semiwinter and spring wheats.

Ammonium thiocyanate was decidely toxic as a seed treatment, the stands being reduced and the vigor lessened even though heads were produced. In the germination trials, ammonium thiocyante reduced germination and the growth of sprouts; it also reduced the length of roots in Kanred and White Winter wheat. Ethyl bromide was slightly toxic to root and sprout growth of White Winter wheat and reduced the germination of this variety markedly as compared with its effects on other varieties.

A difference in time of heading was noted between wet and dry checks. Those soaked in water were from one to three days earlier than those not so treated. The incubation of chemically treated seeds in the germinator for 20 hours before seeding increased the number of heads of spring-planted winter wheats.

Germinating wheat grains survived 12 days of constant freezing at −12° C. Freezing the germinating grains did not break the vegetative dormant period of winter-wheat plants subsequently produced. Alternate freezing and thawing was more detrimental to seedling growth than continuous freezing.

Marquis, a nonwinter hardy spring wheat, survived the low temperatures of the freezing trials much better than did Kanred, a fairly hardy winter wheat. Germination after exposure to low temperature did not indicate the comparative winter hardiness of these two varieties.

Winter wheats showed an individual reaction to time of planting when subsequent growth was considered. Kharkof matured when sown later than the last date of planting for normal heading of Hybrid 128. For late seasons the critical date of planting for heading is correspondingly later.

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FURTHER STUDIES OF THE ENERGY METABOLISM OF CATTLE IN RELATION TO THE PLANE OF NUTRITION [1]

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INTRODUCTION

The objects of the research herein reported were to confirm or disprove the results of an earlier investigation (*4*)[3] and to extend the study to include higher planes of nutrition than were reached in the first series of experiments. This second year's work seemed especially desirable for the reason that the results obtained with the two animals used as subjects in the first year's experiments agreed so very closely as to raise a question as to the extent to which the data were fundamentally significant or were determined by the method of experimentation and rigidity of control.

PLAN OF EXPERIMENTATION

The outline of experiments, as set forth in Table 1, provided for a duplicate series of balances of matter and energy, at seven planes of nutrition between fast and three times the energy maintenance requirement, with two steers as subjects. Only one of the two animals, however, would eat as much as three times maintenance.

The general method of experimentation was the same as in the earlier study, except that the sequence of the experimental treatments was changed.

TABLE 1.—*Schedule of experimentation, daily rations, and live weights of animals*

Period No.	Steer No.	Preliminary feeding period on experimental rations	Total digestion period	Calorimeter period	Plane of nutrition	Rations fed daily		Average live weight of animals
						Roughage (alfalfa hay)	Concentrate (corn meal)	
						Kgm.	*Kgm.*	*Kgm.*
1	60	Sept. 23–Oct. 2	Oct. 3–21	Oct. 18–21	Maintenance	1.574	1.626	310.9
2	57	Oct. 7–16	Oct. 17–Nov. 4	Nov. 1–4	do	1.716	1.774	359.6
3	60	Oct. 21–30	Oct. 31–Nov. 18	Nov. 15–18	One and a half times maintenance.	2.362	2.440	332.9
4	57	Nov. 5–13	Nov. 14–Dec. 2	Nov. 29–Dec. 2	do	2.574	2.662	384.3
5	60	Nov. 18–27	Nov. 28–Dec. 16	Dec. 13–16	Two times maintenance.	3.190	3.290	358.4
6	57	Dec. 3–11	Dec. 12–31	Dec. 28–31	do	3.480	3.584	403.1
7	60	Dec. 21–25	Dec. 26–Jan. 13	Jan. 10–13	Two and a half times maintenance.	4.180	4.310	391.8
8	57	Jan. 3–8	Jan. 9–27	Jan. 24–27	do	4.496	4.636	443.7
9	60	Jan. 18–22	Jan. 23–Feb. 10	Feb. 7–10	Three times maintenance.	5.300	5.470	426.6
10	57	Feb. 4–8	Feb. 9–Mar. 2	Feb. 28–Mar. 2	One-half maintenance	.958	.988	398.3
11	60	Feb. 16–22	Feb. 23–Mar. 16	Mar. 13–16	do	.948	.978	381.0
12	57	Mar. 4–11	Mar. 12–30	Mar. 27–30	Maintenance (alfalfa hay alone).	5.724	------	425.6
13	60	Mar. 16–25	Mar. 26–Apr. 13	Apr. 10–13	do	5.578	------	412.1
14	57	------	------	Apr. 17–21	Fast	------	------	------
15	60	------	------	May 1–5	do	------	------	------

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[2] The authors acknowledge their grateful indebtedness to the Department of Dairy Husbandry of this college for cooperation in the conduct of this series of experiments through the contribution of the efficient services of Paul S. Williams.
[3] Reference is made by number (italic) to the "Literature cited," p. 78.

During the first 11 periods the rations were composed of approximately equal parts by weight (dry-matter basis) of corn meal and alfalfa hay. In periods 1 and 2 the animals were fed at a plane of maintenance, or energy equilibrium, the succeeding periods following in regular order of increasing rates of feeding, to period 9, in which approximately three times the maintenance requirement of feed was given. The rate of feeding was then decreased, in periods 10 and 11, to half of the maintenance requirement; and in the last of the feeding periods, 12 and 13, a maintenance ration of alfalfa hay alone was fed.

In periods 14 and 15 no feed was given, these being the final periods of fast used to establish the base values from which to reckon the effects of feeding at the several planes of nutrition.

The periods on alfalfa hay alone (12 and 13) were included to provide for the determination of the net-energy value of the hay by itself, and to make possible, in connection with periods 1 and 2, during which the mixed ration was fed, the computation, by difference, of the net-energy value of the corn alone.

In accordance with the standard procedure of this institute the periods of fast followed periods of maintenance, thus providing for a uniform influence of previous feeding on the heat production of fast.

The alfalfa hay used was of the same lot as in the earlier study, but the corn was of different lots which were purchased from time to time during the progress of the experiments. The hay was fed cut, as usual, for convenience in sampling and feeding; and the corn was finely ground.

Each experimental feeding period comprised, as usual, a 10-day preliminary feeding on the experimental ration and an 18-day metabolism period during which urine and feces were collected. The last three days constituted the period of respiration calorimetric measurements.

The heat production of fast was measured during the fourth, fifth, sixth, and seventh days after the withdrawal of feed, but the heat production of the fourth day was used as the base value, to represent the energy metabolism of fast, in the computation of results. This datum will be discussed later.

The details of experimental technic were, with slight exceptions to be noted, the standard procedures of this institute, including the latest revisions of method as enumerated in the earlier paper (*4, p. 257*).

As in the previous year's work, the value used for the heat production in each of the feeding periods was the average of the direct heat measurement and the heat production as computed by the balance method, but, differing from the practice of the year before, the value used for the heat production of fast was computed from the gravimetrically determined carbon dioxide by the use of the assumed respiratory quotient of 0.707. The necessity and the justification for the assumption of this respiratory quotient will be discussed later.

EXPERIMENTAL SUBJECTS

The steers used, which were designated Nos. 57 and 60, were purchased at the Pittsburgh, Pa., market. They were roan Shorthorns, approximately 2 years of age, and of unknown history. Their average live weights in periods 1 and 2 were 310.9 and 359.6 kgm., respectively.

Steer No. 57 reached an average weight of 443.7 kgm. in period 8, and steer No. 60, an average weight of 426.6 kgm. in period 9, at planes of nutrition of two and a half and three times maintenance, respectively.

The extent to which these increases in live weight during rise in the plane of nutrition, in the course of these experiments, involved increase in the weight and in the dry substance of the contents of the alimentary tract, is not definitely known, but is important in relation to the computation of the energy expense of food utilization—the heat increment—as will be explained later. As a result of recent informal observations, it is the belief of the writers that the animal tends to maintain the gross weight of the contents of the alimentary tract constant, by means of water, in spite of extensive changes in the plane of nutrition, the concentration of the total dry matter in the contents of the alimentary tract decreasing prominently with the lowering of the plane of nutrition.

In computing the heat increment, the difference in feed between two periods is related to the difference in heat production between the same periods. However, since the live weight will naturally differ in the two experimental periods at different planes of nutrition, it is necessary to compute the heat production in both periods to the basis of the same live weight, so that the heat increment, which is defined as the total increase in heat production incident to the utilization of food, will not be affected by a difference in the energy expense of maintenance.

No accurate method is known for taking into account, in this relation, the difference in the live weight, or the difference in the quantity and the composition of the contents of the alimentary tract of the animals, in the experimental periods at the different planes of nutrition which are compared. It is therefore desirable that the live weights of the animals, in the periods compared, should be as nearly the same as practicable, so that the correction of the heat production for difference in live weights may be as small as possible, since this correction involves elements of error.

It was found in the course of the organization of the results that the sequence of experimental periods was unfortunate in two respects, (1) that the maintenance periods on hay alone (12 and 13) were so widely separated from the maintenance periods on hay and corn (1 and 2), since the determination of the net-energy value of the corn depended on the comparison of the heat production of these periods; and (2) since in computing the heat increments between fast and the several levels of feeding, the smallest differences in feed were associated with the greatest differences in the live weight of the animals, thus leading to the maximum possible exaggeration of any such error as exists in the correction of the heat production, as observed, to the basis of a uniform live weight.

This element of error is believed not to be extensive, and is not observable in the curve of heat production, but seems prominently to affect some of the derived values, especially the net-energy values of the mixed ration and of the grain, for maintenance, and of the individual feeds, for body increase, as computed by the heat-increment-proportional method used in the former study. From this point of view the sequence of experimental treatments in the previous study was much more satisfactory.

It appears, however, that the gain from this unfortunate situation is immeasurably greater than the loss, since a new and important requirement in the planning of experiments for the determination of net-energy values of feeds has been brought clearly to light. This matter will be discussed further in relation to the problem of determining net-energy values.

TABLE 2.—*Digestibility of rations*

Period No.	Steer No.	Item	Dry matter	Organic matter	Crude protein	Crude fiber	Ether extract	N-free extract	Carbon	Energy	Nitrogen
		Salt.....grams..	30								
		Alfalfa hay.....do....	1,420	1,290.2	208.4	493.1	20.0	568.8	652.0	6,294.0	33.3
1	60	Corn meal.....do....	1,408	1,385.8	139.5	31.4	57.5	1,157.3	649.2	6,335.6	22.3
		Total fed.....do....	2,858	2,676.0	347.9	524.5	77.5	1,726.1	1,301.2	12,629.6	55.6
		Feces.....do....	694	627.7	111.5	291.0	27.4	197.8	344.9	3,461.4	17.8
		Digestibility (per cent)	75.7	76.5	68.0	44.5	64.6	88.5	73.5	72.6	68.0
		Salt.....grams..	30								
		Alfalfa hay.....do....	1,546	1,404.7	226.9	536.8	21.7	619.3	709.8	6,852.5	36.3
2	57	Corn meal.....do....	1,539	1,514.7	152.5	34.3	62.9	1,265.0	709.6	6,925.0	24.4
		Total fed.....do....	3,115	2,919.4	379.4	571.1	84.6	1,884.3	1,419.4	13,777.5	60.7
		Feces.....do....	750	676.1	111.6	310.5	31.2	222.8	362.8	3,639.2	17.9
		Digestibility (per cent)	75.9	76.8	70.6	45.6	63.1	88.2	74.4	73.6	70.5
		Salt.....grams..	30								
		Alfalfa hay.....do....	2,134	1,938.9	313.2	741.0	30.0	854.8	979.8	9,458.7	50.1
3	60	Corn meal.....do....	2,103	2,070.1	207.8	46.4	87.2	1,728.6	974.0	9,473.8	33.2
		Total fed.....do....	4,267	4,009.0	521.0	787.4	117.2	2,583.4	1,953.8	18,932.5	83.3
		Feces.....do....	1,132	1,030.9	187.5	409.9	45.5	387.7	550.1	5,465.9	30.0
		Digestibility (per cent)	73.5	74.3	64.0	47.9	61.2	85.0	71.8	71.1	64.0
		Salt.....grams..	30								
		Alfalfa hay.....do....	2,319	2,107.0	340.4	805.2	32.6	928.9	1,064.8	10,278.7	54.5
4	57	Corn meal.....do....	2,293	2,257.1	226.6	50.6	95.1	1,884.8	1,062.0	10,329.7	36.3
		Total fed.....do....	4,642	4,364.1	566.9	855.8	127.7	2,813.7	2,126.8	20,608.4	90.8
		Feces.....do....	1,118	1,012.1	190.0	418.3	52.0	351.8	549.5	5,483.6	30.4
		Digestibility (per cent)	75.9	76.8	66.5	51.1	59.3	87.5	74.2	73.4	66.5
		Salt.....grams..	30								
		Alfalfa hay.....do....	2,866	2,604.0	420.6	995.1	40.3	1,148.0	1,315.9	12,703.3	67.3
5	60	Corn meal.....do....	2,838	2,793.6	280.4	62.7	117.7	2,332.8	1,314.5	12,784.9	44.9
		Total fed.....do....	5,734	5,397.6	701.0	1,057.8	158.0	3,480.8	2,630.4	25,488.2	112.2
		Feces.....do....	1,551	1,413.7	261.2	564.3	57.8	530.5	751.9	7,484.4	41.8
		Digestibility (per cent)	73.0	73.8	62.7	46.7	63.4	84.8	71.4	70.6	62.7
		Salt.....grams..	30								
		Alfalfa hay.....do....	3,144	2,856.6	461.4	1,091.7	44.2	1,259.3	1,443.6	13,935.5	73.8
6	57	Corn meal.....do....	3,089	3,040.6	305.2	68.2	128.1	2,539.1	1,430.7	13,915.6	48.8
		Total fed.....do....	6,263	5,897.2	766.6	1,159.9	172.3	3,798.4	2,874.3	27,851.1	122.6
		Feces.....do....	1,637	1,484.2	273.2	589.6	79.9	541.5	803.8	8,029.3	43.7
		Digestibility (per cent)	73.9	74.8	64.4	49.2	53.6	85.7	72.0	71.2	64.4
		Salt.....grams..	30								
		Alfalfa hay.....do....	3,793	3,446.2	556.6	1,317.0	53.3	1,519.3	1,741.6	16,812.1	89.1
7	60	Corn meal.....do....	3,727	3,668.6	368.3	82.3	154.6	3,063.5	1,726.2	16,789.8	58.9
		Total fed.....do....	7,550	7,114.8	924.9	1,399.3	207.9	4,582.8	3,467.8	33,601.9	148.0
		Feces.....do....	2,117	1,922.9	365.3	752.8	78.1	726.7	1,040.8	10,337.0	58.5
		Digestibility (per cent)	72.0	73.0	60.5	46.2	62.4	84.1	70.0	69.2	60.5
		Salt.....grams..	30								
		Alfalfa hay.....do....	4,051	3,680.7	594.5	1,406.6	57.0	1,622.6	1,860.0	17,955.7	95.1
8	57	Corn meal.....do....	4,006	3,944.3	397.6	91.5	170.3	3,284.9	1,855.9	18,069.9	63.6
		Total fed.....do....	8,087	7,625.0	992.1	1,498.1	227.3	4,907.5	3,715.9	36,025.6	158.7
		Feces.....do....	2,248	2,049.5	377.0	825.4	84.1	763.1	1,096.3	10,879.8	60.3
		Digestibility (per cent)	72.2	73.1	62.0	44.9	63.0	84.5	70.5	69.8	62.0
		Salt.....grams..	30								
		Alfalfa hay.....do....	4,763	4,327.6	699.0	1,653.8	67.0	1,907.8	2,186.9	21,111.5	111.8
9	60	Corn meal.....do....	4,726	4,653.2	469.1	108.0	200.9	3,875.3	2,189.5	21,317.6	75.0
		Total fed.....do....	9,519	8,980.8	1,168.1	1,761.8	267.9	5,783.1	4,376.4	42,429.1	186.8
		Feces.....do....	2,926	2,654.3	476.8	1,026.0	97.4	1,054.1	1,416.3	13,958.5	76.3
		Digestibility (per cent)	69.3	70.4	59.2	41.8	63.6	81.8	67.6	67.1	59.2
		Salt.....grams..	30								
		Alfalfa hay.....do....	871	791.4	127.8	302.4	12.2	348.9	399.9	3,860.6	20.5
10	57	Corn meal.....do....	829	815.4	80.3	20.2	34.6	680.4	383.0	3,734.9	12.8
		Total fed.....do....	1,730	1,606.8	208.1	322.6	46.8	1,029.3	782.9	7,595.5	33.3
		Feces.....do....	424	379.4	65.5	185.7	14.8	113.4	207.7	2,072.0	10.5
		Digestibility (per cent)	75.5	76.4	68.5	42.4	68.4	89.0	73.5	72.7	68.5
		Salt.....grams..	30								
		Alfalfa hay.....do....	862	783.2	126.5	299.3	12.1	345.3	395.8	3,820.7	20.2
11	60	Corn meal.....do....	819	805.6	79.3	19.9	34.2	672.2	378.4	3,689.8	12.7
		Total fed.....do....	1,711	1,588.8	205.8	319.2	46.3	1,017.5	774.2	7,510.5	32.9
		Feces.....do....	436	395.6	72.6	184.1	18.2	120.6	213.4	2,128.2	11.6
		Digestibility (per cent)	74.5	75.1	64.7	42.3	60.7	88.1	72.4	71.7	64.7
		Salt.....grams..	30								
		Alfalfa hay.....do....	5,155	4,683.7	756.5	1,789.8	72.5	2,064.8	2,366.9	22,849.0	121.0
12	57	Feces.....do....	2,050	1,860.8	257.9	956.4	72.8	573.8	1,005.3	9,905.0	41.3
		Digestibility (per cent)	60.5	60.3	65.9	46.6	.....	72.2	57.5	56.7	65.9
		Salt.....grams..	30								
		Alfalfa hay.....do....	5,013	4,554.7	735.7	1,740.6	70.5	2,008.0	2,301.7	22,219.6	117.7
13	60	Feces.....do....	2,077	1,879.3	263.9	943.3	82.5	589.6	1,013.3	10,002.5	42.2
		Digestibility (per cent)	58.0	58.8	64.1	45.8	.....	70.6	56.0	55.0	64.1

TABLE 3.—*Carbon dioxide, water vapor, and methane eliminated per day*

Steer No.	Period No.	Calorimeter day	CO_2	C as CO_2	H_2O	CH_4	C as CH_4
			Grams	*Grams*	*Grams*	*Grams*	*Grams*
60	1	First	2,945.9	803.3	2,759.3	75.9	56.8
		Second					
		Third	2,922.7	797.0	2,718.4	83.1	62.2
		Average	2,934.3	800.2	2,738.9	79.5	59.5
57	2	First	3,271.5	892.1	3,761.7	96.0	71.8
		Second	3,271.9	892.2	3,536.9	94.1	70.4
		Third	3,196.7	871.8	3,435.0	93.8	71.7
		Average	3,246.7	885.4	3,577.9	95.3	71.3
60	3	First	3,749.9	1,022.6	4,100.6		
		Second	3,761.6	1,025.8	4,039.0	110.1	82.4
		Third	3,740.0	1,019.9	4,294.6	112.1	83.9
		Average	3,750.5	1,022.8	4,144.7	111.4	83.2
57	4	First	4,122.4	1,124.2	5,471.0	136.5	102.2
		Second	4,126.5	1,125.3	5,193.4	132.7	99.3
		Third	4,064.8	1,108.4	5,044.2	131.4	98.3
		Average	4,104.5	1,119.3	5,236.2	133.5	99.9
60	5	First	4,847.9	1,322.0	7,659.0	156.8	117.3
		Second	4,837.3	1,319.1	7,086.5	155.8	116.6
		Third	4,897.2	1,335.5	7,059.2	156.4	117.1
		Average	4,860.8	1,325.5	7,268.2	156.3	117.0
57	6	First			8,809.9	170.4	127.5
		Second	5,223.4	1,424.4	8,746.7	173.7	130.0
		Third	5,396.7	1,471.7	8,987.1	181.0	135.4
		Average	5,310.1	1,448.1	8,847.9	175.0	131.0
60	7	First	6,279.7	1,712.5	11,596.7	186.8	139.8
		Second	6,130.9	1,671.9	10,872.0	185.2	138.6
		Third	6,142.7	1,675.1	10,493.9	194.2	145.4
		Average	6,184.4	1,686.5	10,987.5	188.7	141.3
57	8	First	6,267.1	1,709.0	10,459.9	212.4	159.0
		Second					
		Third	6,678.6	1,821.3	11,090.5	226.8	169.8
		Average	6,472.9	1,765.2	10,775.2	219.6	164.4
60	9	First	7,276.1	1,984.2	8,151.3	232.7	174.1
		Second	7,290.0	1,988.0	8,775.6	228.3	170.9
		Third	7,326.3	1,997.9	8,810.4	230.2	172.3
		Average	7,297.5	1,990.0	8,579.1	230.4	172.4
57	10	First	2,891.1	788.4	3,831.4	57.4	43.0
		Second	2,845.3	775.9	3,762.3	55.9	41.8
		Third	2,848.9	776.9	3,655.9	54.1	40.5
		Average	2,861.8	780.4	3,749.9	55.8	41.8
60	11	First	2,771.5	755.8	2,894.1	56.4	42.2
		Second	2,717.2	741.0	2,850.3	55.4	41.4
		Third	2,667.7	727.5	2,815.5	54.0	40.4
		Average	2,718.8	741.4	2,853.3	55.3	41.3
57	12	First	4,066.8	1,109.0	4,930.0	121.3	90.8
		Second	4,009.4	1,093.4	4,805.0	118.1	88.4
		Third	3,979.9	1,085.3	4,867.2	118.7	88.8
		Average	4,018.7	1,095.9	4,867.4	119.4	89.3
60	13	First	3,860.8	1,052.8	4,667.7	109.5	81.9
		Second	3,899.0	1,063.2	4,763.5	109.9	82.2
		Third	3,892.5	1,061.5	4,949.4	110.0	82.3
		Average	3,884.1	1,059.2	4,793.5	109.8	82.1

TABLE 4.—*Energy of the urine and of the protein corrected for the incomplete oxidation of protein gained or lost*

Period No.	Balance of N	Correction (N × 7.45)	Energy of urine		Energy of protein	
			Uncorrected for N equilibrium	Corrected	Uncorrected grams protein (× 5.7)	Corrected
	Grams	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
1	−2.6	−19.4	581.7	562.3	88.9	69.5
2	−4.9	−36.5	659.9	623.4	167.6	131.1
3	+13.5	+100.6	652.8	753.4	461.7	361.1
4	+15.5	+115.5	758.5	874.0	530.1	414.6
5	+20.7	+154.2	862.3	1,016.5	707.9	553.7
6	+14.6	+138.6	984.8	1,123.4	636.1	497.5
7	+23.0	+171.4	1,108.4	1,279.8	786.6	615.2
8	+19.1	+142.3	1,217.8	1,360.1	653.2	510.9
9	+22.1	+164.6	1,335.6	1,500.2	755.8	591.2
10	−16.9	−125.9	512.5	386.6	578.0	452.1
11	−17.8	−132.6	506.6	374.0	608.8	476.2
12	+1.0	+7.5	1,155.1	1,162.6	34.2	26.7
13	+2.9	+21.6	1,072.6	1,094.2	99.2	77.6

TABLE 5.—*Balance of matter and energy per day*

PERIOD 1, STEER NO. 60

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Calories*
Income:					
Alfalfa hay	1,420	161	33.3	652.0	6,294.0
Corn meal	1,408	216	22.3	649.2	6,335.6
Water		5,743			
Total	2,828	6,120	55.6	1,301.2	12,629.6
Outgo:					
Feces	694	1,795	17.8	344.9	3,461.4
Urine		2,691	40.4	66.8	562.3
Methane	79.5			59.5	1,060.8
Carbon dioxide	2,934.3			800.2	
Water vapor		2,739			
Metabolizable: Income minus urine, feces, and methane					7,545.1
Body balances:					
Fat	+49.7			+38.0	+472.2
Protein	−15.6		−2.6	−8.2	−69.5
Water		−1,105			
Computed heat production					7,142.4
Observed heat production					7,252.9

PERIOD 2, STEER NO. 57

Income:					
Alfalfa hay	1,546	195	36.3	709.8	6,852.5
Corn meal	1,539	245	24.4	709.6	6,925.0
Water		6,850			
Total	3,085	7,290	60.7	1,419.4	13,777.5
Outgo:					
Feces	750	2,968	17.9	362.8	3,639.2
Urine		3,780	47.7	72.6	623.4
Methane	95.3			71.3	1,271.7
Carbon dioxide	3,246.7			885.4	
Water vapor		3,578			
Metabolizable: Income minus urine, feces, and methane					8,243.2
Body balances:					
Fat	+55.8			+42.7	+530.1
Protein	−29.4		−4.9	−15.4	−131.1
Water		−3,036			
Computed heat production					7,844.2
Observed heat production					7,908.7

TABLE 5.—*Balance of matter and energy per day*—Continued

PERIOD 3, STEER NO. 60

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Calories*
Income:					
Alfalfa hay	2,134	233	50.1	979.8	9,458.7
Corn meal	2,103	340	33.2	974.0	9,473.8
Water		13,237			
Total	4,237	13,810	83.3	1,953.8	18,932.5
Outgo:					
Feces	1,132	5,821	30.0	550.1	5,465.9
Urine		5,118	39.8	74.7	742.9
Methane	111.1			83.2	1,482.5
Carbon dioxide	3,750.5			1,022.8	
Water vapor		4,145			
Metabolizable: Income minus urine, feces, and methane					11,241.2
Body balances:					
Fat	+235.8			+180.4	+2,240.1
Protein	+81.0		+13.5	+42.6	+361.1
Water		−1,274			
Computed heat production					8,640.0
Observed heat production					8,821.4

PERIOD 4, STEER NO. 57

Income:					
Alfalfa hay	2,319	267	54.5	1,064.8	10,278.7
Corn meal	2,293	381	36.3	1,062.0	10,329.7
Water		8,143			
Total	4,612	8,791	90.8	2,126.8	20,608.4
Outgo:					
Feces	1,118	4,276	30.4	549.5	5,483.6
Urine		5,152	44.9	86.8	874.0
Methane	133.5			99.9	1,781.4
Carbon dioxide	4,104.5			1,119.3	
Water vapor		5,236			
Metabolizable: Income minus urine, feces, and methane					12,469.4
Body balances:					
Fat	+290.7			+222.4	+2,761.7
Protein	+93.0		+15.5	+48.9	+414.6
Water		−5,873			
Computed heat production					9,293.1
Observed heat production					9,493.3

PERIOD 5, STEER NO. 60

Income:					
Alfalfa hay	2,866	349	67.3	1,315.9	12,703.3
Corn meal	2,838	465	44.9	1,314.5	12,784.9
Water		15,623			
Total	5,704	16,437	112.2	2,630.4	25,488.2
Outgo:					
Feces	1,551	5,683	41.8	751.9	7,484.4
Urine		4,840	49.7	99.2	1,016.5
Methane	156.3			117.0	2,085.7
Carbon dioxide	4,860.8			1,325.5	
Water vapor		7,268			
Metabolizable: Income minus urine, feces, and methane					14,901.6
Body balances:					
Fat	+354.9			+271.5	+3,371.6
Protein	+124.2		+20.7	+65.3	+553.7
Water		−1,354			
Computed heat production					10,976.3
Observed heat production					11,156.9

TABLE 5.—*Balance of matter and energy per day*—Continued

PERIOD 6, STEER NO. 57

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Calories*
Income:					
Alfalfa hay	3,144	342	73.8	1,443.6	13,935.5
Corn meal	3,089	505	48.8	1,430.7	13,915.6
Water		17,490			
Total	6,233	18,337	122.6	2,874.3	27,851.1
Outgo:					
Feces	1,637	5,630	43.7	803.8	8,029.3
Urine		6,234	60.3	111.7	1,123.4
Methane	175.0			131.0	2,335.2
Carbon dioxide	5,310.1			1,448.1	
Water vapor		8,848			
Metabolizable: Income minus urine, feces, and methane					16,363.2
Body balances:					
Fat	+419.7			+321.1	+3,987.2
Protein	+111.6		+18.6	+58.6	+497.5
Water		−2,375			
Computed heat production					11,878.5
Observed heat production					11,851.2

PERIOD 7, STEER NO. 60

Income:					
Alfalfa hay	3,793	393	89.1	1,741.6	16,812.1
Corn meal	3,727	596	58.9	1,726.2	16,789.8
Water		20,813			
Total	7,520	21,802	148.0	3,467.8	33,601.9
Outgo:					
Feces	2,117	7,685	58.5	1,040.8	10,337.0
Urine		5,858	66.5	126.6	1,279.8
Methane	188.7			141.3	2,518.0
Carbon dioxide	6,184.4			1,686.5	
Water vapor		10,988			
Metabolizable: Income minus urine, feces, and methane					19,467.1
Body balances:					
Fat	+522.9			+400.1	+4,967.6
Protein	+138.0		+23.0	+72.5	+615.2
Water		−2,729			
Computed heat production					13,884.3
Observed heat production					13,976.4

PERIOD 8, STEER NO. 57

Income:					
Alfalfa hay	4,051	*771	95.1	1,860.0	17,955.7
Corn meal	4,006	*948	63.6	1,855.9	18,069.9
Water		25,195			
Total	8,057	26,914	158.7	3,715.9	36,025.6
Outgo:					
Feces	2,248	7,926	60.3	1,096.3	10,879.8
Urine		6,774	79.3	140.8	1,360.1
Methane	219.6			164.4	2,930.3
Carbon dioxide	6,472.9			1,765.2	
Water vapor		10,775			
Metabolizable: Income minus urine, feces, and methane					20,855.4
Body balances:					
Fat	+639.1			+489.0	+6,071.5
Protein	+114.6		+19.1	+60.2	+510.9
Water		+1,439			
Computed heat production					14,273.0
Observed heat production					14,408.2

* Includes 313 grams of water added to refused feed fed back.

TABLE 5.—*Balance of matter and energy per day*—Continued

PERIOD 9, STEER NO. 60

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Calories*
Income:					
Alfalfa hay	4,763	837	111.8	2,186.9	21,111.5
Corn meal	4,726	744	75.0	2,189.5	21,317.6
Water		22,817			
Total	9,489	24,098	186.8	4,376.4	42,429.1
Outgo:					
Feces	2,926		76.3	1,416.3	13,958.5
Urine			88.4	150.4	1,500.2
Methane	230.4			172.4	3,074.5
Carbon dioxide	7,297.5			1,990.0	
Water vapor		8,579			
Metabolizable: Income minus urine, feces, and methane					23,895.9
Body balances:					
Fat	+754.9			+577.6	+7,171.6
Protein	+132.6		+22.1	+69.7	+591.2
Water					
Computed heat production					16,133.1
Observed heat production					

PERIOD 10, STEER NO. 57

Income:					
Alfalfa hay	871	87	20.5	399.9	3,860.6
Corn meal	829	151	12.8	383.0	3,734.9
Water		9,300			
Total	1,700	9,538	33.3	782.9	7,595.5
Outgo:					
Feces	424	1,623	10.5	207.7	2,072.0
Urine		3,421	39.7	55.8	386.6
Methane	55.8			41.8	744.6
Carbon dioxide	2,861.8			780.4	
Water vapor		3,750			
Metabolizable: Income minus urine, feces, and methane					4,392.3
Body balances:					
Fat	−326.1			−249.5	−3,098.0
Protein	−101.4		−16.9	−53.3	−452.1
Water		+744			
Computed heat production					7,942.4
Observed heat production					7,939.1

PERIOD 11, STEER NO. 60

Income:					
Alfalfa hay	862	90	20.2	395.8	3,820.7
Corn meal	819	166	12.7	378.4	3,689.8
Water		9,697			
Total	1,681	9,953	32.9	774.2	7,510.5
Outgo:					
Feces	436	1,905	11.6	213.4	2,128.2
Urine		3,780	39.1	53.3	374.0
Methane	55.3			41.3	737.9
Carbon dioxide	2,718.8			741.4	
Water vapor		2,853			
Metabolizable: Income minus urine, feces, and methane					4,270.4
Body balances:					
Fat	−286.4			−219.1	−2,720.8
Protein	−106.8		−17.8	−56.1	−476.2
Water		+1,415			
Computed heat production					7,467.4
Observed heat production					7,476.2

TABLE 5.—*Balance of matter and energy per day*—Continued

PERIOD 12, STEER NO. 57

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Calories*
Income:					
Alfalfa hay	5,155	577	121.0	2,366.9	22,849.0
Water		18,067			
Total	5,155	18,644	121.0	2,366.9	22,849.0
Outgo:					
Feces	2,050	8,417	41.3	1,005.3	9,905.0
Urine		7,350	78.7	135.0	1,162.6
Methane	119.4			89.3	1,593.3
Carbon dioxide	4,018.7			1,095.9	
Water vapor		4,867			
Metabolizable: Income minus urine, feces, and methane					10,188.1
Body balances:					
Fat	+49.9			+38.2	+474.1
Protein	+6.0		+1.0	+3.2	+26.7
Water		−1,990			
Computed heat production					9,687.3
Observed heat production					9,953.7

PERIOD 13, STEER NO. 60

Income:					
Alfalfa hay	5,013	569	117.7	2,301.7	22,219.6
Water		17,155			
Total	5,013	17,724	117.7	2,301.7	22,219.6
Outgo:					
Feces	2,077	8,702	42.2	1,013.3	10,002.5
Urine		7,156	72.6	130.8	1,094.2
Methane	109.8			82.1	1,465.2
Carbon dioxide	3,884.1			1,059.2	
Water vapor		4,794			
Metabolizable: Income minus urine, feces, and methane					9,657.7
Body balances:					
Fat	+9.4			+7.2	+89.3
Protein	+17.4		+2.9	+9.1	+77.6
Water		−2,928			
Computed heat production					9,490.8
Observed heat production					9,790.1

TABLE 6.—*Heat emission and heat production per day*

Steer No.	Period No.	Calorimeter days	Sub-period No.	Heat emission			Corrections		Heat production
				By radiation and conduction	As latent heat of water vapor	Total	Body gain	Platform	
				Calories	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
60	1		1	2,845.2					
			2	2,840.7					
		First		5,685.9	1,619.8	7,305.7			
			5	2,820.0					
			6	2,829.7					
		Third		5,649.7	1,595.6	7,245.3			
		Daily average		5,667.8	1,607.7	7,275.5	−22.6	0	7,252.9
57	2		1	3,012.2					
			2	2,976.4					
		First		5,988.6	2,208.0	8,196.6			
			3	2,886.9					
			4	2,960.0					
		Second		5,846.9	2,076.2	7,923.1			
			5	2,874.3					
			6	2,873.4					
		Third		5,747.7	2,006.2	7,753.9			
		Daily average		5,861.1	2,096.8	7,957.9	−63.7	+14.5	7,908.7
60	3		1	3,028.8					
			2	3,273.3					
		First		6,302.1	2,407.0	8,709.1			
			3	3,127.3					
			4	3,298.1					
		Second		6,425.9	2,371.0	8,796.9			
			5	3,166.3					
			6	3,340.8					
		Third		6,507.1	2,521.0	9,028.1			
		Daily average		6,411.7	2,433.0	8,844.7	−23.3	0	8,821.4
57	4		1	3,045.1					
			2	3,287.2					
		First		6,332.3	3,211.6	9,543.9			
			3	3,165.8					
			4	3,442.9					
		Second		6,608.7	3,048.6	9,657.3			
			5	3,327.2					
			6	3,314.5					
		Third		6,641.7	2,961.0	9,602.7			
		Daily average		6,527.6	3,073.7	9,601.3	−121.3	+13.3	9,493.3
60	5		1	3,495.2					
			2	3,768.6					
		First		7,263.8	3,915.3	11,179.1			
			3	3,425.8					
			4	3,744.0					
		Second		7,169.8	3,967.2	11,137.0			
			5	3,401.0					
			6	3,793.0					
		Third		7,194.0	4,029.8	11,223.8			
		Daily average		7,209.2	3,970.8	11,180.0	−23.1	0	11,156.9
57	6		1	3,797.9					
			2	4,094.6					
		First		7,892.5	3,838.4	11,730.9			
			3	3,810.8					
			4	4,148.4					
		Second		7,959.2	3,801.2	11,760.4			
			5	4,012.0					
			6	4,223.4					
		Third		8,235.4	3,942.4	12,177.8			
		Daily average		8,029.0	3,860.7	11,889.7	−44.9	+6.4	11,851.2

TABLE 6.—*Heat emission and heat production per day*—Continued

Steer No.	Period No.	Calorimeter days	Sub-period No.	Heat emission			Corrections		Heat production
				By radiation and conduction	As latent heat of water vapor	Total	Body gain	Platform	
				Calories	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
			1	4,504.9					
			2	4,926.3					
		First		9,431.2	4,987.2	14,418.4			
			3	4,306.1					
			4	4,561.2					
60	7	Second		8,867.3	4,884.5	13,751.8			
			5	4,335.8					
			6	4,734.3					
		Third		9,070.1	4,807.4	13,877.5			
		Daily average		9,122.9	4,893.0	14,015.9	−54.0	+14.5	13,976.4
			1	4,606.0					
			1	4,664.0					
		First		9,270.0	4,722.8	13,992.8			
			3	4,549.9					
			4	5,014.1					
57	8	Second		9,564.0	4,705.2	14,269.2			
			5	4,827.4					
			6	5,150.4					
		Third		9,977.8	4,850.8	14,828.6			
		Daily average		9,603.9	4,759.6	14,363.5	+44.7	0	14,408.2
			1	2,868.1					
			2	2,755.5					
		First		5,623.6	2,250.9	7,874.5			
			3	2,825.1					
			4	2,848.5					
57	10	Second		5,673.6	2,210.3	7,883.9			
			5	2,941.7					
			6	2,893.9					
		Third		5,835.6	2,147.8	7,983.4			
		Daily average		5,710.9	2,203.0	7,913.9	+10.7	+14.5	7,939.1
			1	2,849.0					
			2	2,917.3					
		First		5,766.3	1,701.8	7,468.1			
			3	2,974.2					
			4	2,953.3					
60	11	Second		5,927.5	1,676.0	7,603.5			
			5	2,734.4					
			6	2,890.3					
		Third		5,624.7	1,655.5	7,280.2			
		Daily average		5,772.8	1,677.8	7,450.6	+25.6	0	7,476.2
			1	3,327.0					
			2	3,787.1					
		First		7,114.1	2,898.8	10,012.9			
			3	3,325.5					
			4	3,796.6					
57	12	Second		7,122.1	2,825.4	9,947.5			
			5	3,548.9					
			6	3,638.7					
		Third		7,187.6	2,862.0	10,049.6			
		Daily average		7,141.3	2,862.1	10,003.3	−42.6	−7.0	9,953.7
			1	3,505.5					
			2	3,613.3					
		First		7,118.8	2,744.6	9,863.4			
			3	3,375.2					
			4	3,634.3					
60	13	Second		7,009.5	2,801.0	9,810.5			
			5	3,389.0					
			6	3,588.9					
		Third		6,977.9	2,910.2	9,888.1			
		Daily average		7,035.4	2,818.6	9,854.0	−63.9	0	9,790.1

TABLE 7.—*Correction of heat production to a standard day of 12 hours standing and 12 hours lying*

Period No.	Steer No.	Time spent standing	Difference from 12 hours	Factor *	Correction	Observed heat production		Computed heat production	
						Uncorrected	Corrected	Uncorrected	Corrected
		Hours	*Hours*		*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
1	60	7.9	4.1	20.6	+84.5	7,252.9	7,337.4	7,142.4	7,226.9
2	57	8.8	3.2	60.6	+193.9	7,908.7	8,102.6	7,844.2	8,038.1
3	60	5.6	6.4	22.1	+141.4	8,821.4	8,962.8	8,640.0	8,781.4
4	57	7.4	4.6	64.8	+298.1	9,493.3	9,791.4	9,293.1	9,591.2
5	60	5.0	7.0	23.8	+166.6	11,156.9	11,323.5	10,976.3	11,142.9
6	57	6.1	5.9	68.0	+401.2	11,851.2	12,252.4	11,878.5	12,279.7
7	60	6.5	5.5	26.0	+143.0	13,976.4	14,119.4	13,884.3	14,027.3
8	57	8.4	3.6	74.8	+269.3	14,408.2	14,677.5	14,273.0	14,542.3
9	60	8.8	3.2	28.3	+90.6			16,133.1	16,223.7
10	57	10.4	1.6	67.2	+107.5	7,939.1	8,046.6	7,942.4	8,049.9
11	60	8.2	3.8	25.3	+96.1	7,476.2	7,572.3	7,467.4	7,563.5
12	57	7.0	5.0	71.8	+359.0	9,953.7	10,312.7	9,687.3	10,046.3
13	60	7.4	4.6	27.3	+125.6	9,790.1	9,915.7	9,490.8	9,616.4

* Based on CO_2 production during fast, and computed directly in proportion to the live weight.

TABLE 8.—*Urinary nitrogen per 12 hour subperiod, and carbon dioxide, oxygen, and energy equivalents during fast*

Steer No. and period	Average urinary nitrogen per 12-hour subperiod	Carbon dioxide equivalent (N×4.75)	Oxygen equivalent (N×5.94)	Energy equivalent (N×26.51)
	Grams	*Liters*	*Liters*	*Calories*
Steer 57, period 14	19.6	93.1	116.4	519.6
Steer 60, period 15	21.6	102.6	128.3	572.6

TABLE 9.—*Carbon dioxide, water vapor, and methane eliminated per day during fast*

Steer No. and period	Subperiod and day of fast	Carbon dioxide	Water vapor	Methane
		Grams	*Grams*	*Grams*
Steer 57, period 14	Subperiod 1	1,184.55	1,685.35	1.23
	Subperiod 2	1,114.26	1,540.92	.76
	Fourth day	2,298.81	3,226.27	1.99
	Subperiod 3	1,184.90	1,786.91	.28
	Subperiod 4	1,111.62	1,903.18	.61
	Fifth day	2,296.52	3,690.09	.89
	Subperiod 5	1,207.69	2,677.31	.61
	Subperiod 6	1,003.90	1,572.01	1.09
	Sixth day	2,211.59	4,249.32	1.70
	Subperiod 7	1,090.49	1,484.67	.61
	Subperiod 8	1,057.29	1,381.34	.13
	Seventh day	2,147.78	2,866.01	.74
Steer 60, period 15	Subperiod 1	1,081.07	1,813.10	1.35
	Subperiod 2	1,038.26	1,717.03	.55
	Fourth day	2,119.33	3,530.13	1.90
	Subperiod 3	1,047.03	1,462.72	None.
	Subperiod 4	1,032.40	1,371.22	None.
	Fifth day	2,079.43	2,833.94	None.
	Subperiod 5	1,023.04	1,207.83	None.
	Subperiod 6	992.00	1,103.85	None.
	Sixth day	2,015.04	2,311.68	None.
	Subperiod 7	994.95	1,078.52	None.
	Subperiod 8	986.47	1,035.61	None.
	Seventh day	1,981.42	2,114.13	None.

TABLE 10.—*Heat production computed from the CO_2 eliminated, assuming a nonprotein respiratory quotient of 0.707 eliminated*

Steer No., period, and subperiod	Nonprotein CO_2 eliminated	Energy of nonprotein CO_2(6.628)[a]	Energy of protein	Heat production
	Liters	*Calories*	*Calories*	*Calories*
Steer 57, period 14:				
Subperiod 1	501.7	3,325.3	519.6	3,844.9
Subperiod 2	470.3	3,117.1	519.6	3,636.7
Subperiod 3	502.6	3,331.2	519.6	3,850.8
Subperiod 4	469.5	3,111.8	519.6	3,631.4
Subperiod 5	531.4	3,522.1	519.6	4,041.7
Subperiod 6	415.1	2,751.3	519.6	3,270.9
Subperiod 7	458.5	3,038.9	519.6	3,558.5
Subperiod 8	444.5	2,946.1	519.6	3,465.7
Steer 60 period 15:				
Subperiod 1	442.4	2,932.2	572.6	3,504.8
Subperiod 2	426.5	2,826.8	572.6	3,399.4
Subperiod 3	428.0	2,836.8	572.6	3,409.4
Subperiod 4	421.4	2,793.0	572.6	3,365.6
Subperiod 5	424.3	2,812.3	572.6	3,384.9
Subperiod 6	396.9	2,630.7	572.6	3,203.3
Subperiod 7	404.4	2,680.4	572.6	3,253.0
Subperiod 8	395.2	2,619.4	572.6	3,192.0

[a] Calorific value of O_2=4.686 Cals. per liter O_2; calorific value of nonprotein $CO_2=\frac{4.686}{0.707}=6.628$ Cals. per liter CO_2.

TABLE 11.—*Computed heat production[a] of fast corrected to the standard day*

Steer No., period, and day of fast	Average weight of animal, 4th and 7th days	Time spent standing	Difference from standard of time spent standing	Correction[b]	Heat production, uncorrected	Heat production, corrected to standard day
	Kilograms	*Hours*	*Hours*	*Calories*	*Calories*	*Calories*
Steer 57, period 14:						
Fourth day	399.2	6.7	5.3	+356.7	7,481.6	7,838.3
Fifth day		9.5	2.5	+168.3	7,482.2	7,650.5
Sixth day		11.5	.5	+33.7	7,312.6	7,346.3
Seventh day		11.5	.5	+33.7	7,024.2	7,057.9
Steer 60, period 15:						
Fourth day	383.0	6.9	5.1	+129.5	6,904.2	7,033.7
Fifth day		8.6	3.4	+86.4	6,775.0	6,861.4
Sixth day		10.7	1.3	+33.0	6,588.2	6,621.2
Seventh day		9.7	2.3	+58.4	6,445.0	6,503.4

[a] Computed from the CO_2, assuming a nonprotein respiratory quotient of 0.707.
[b] Correction for steer No. 57, 16.86 Cals. per 100 kgm. live weight per hour; for steer No. 60, 6.63 Cals. per hour.

TABLE 12.—*Contents of alimentary tract of steers after fast*

KILOGRAMS DRY MATTER

Steer No.	Contents of—					
	Paunch and reticulum	Omasum	Abomasum	Small intestine	Large intestine	Total, alimentary tract
Steer 57, after 7 days' fast	0.378	0	0.028	0.085	0.036	0.527
Steer 60, after 7 days' fast	.186	0	.035.	.079	.035	.335

KILOGRAMS FRESH SUBSTANCE

Steer 57, after 7 days' fast	10.730	.054	.231	3.696	1.080	15.791
Steer 60, after 7 days' fast	16.032	.130	.518	3.083	1.357	31.129

TABLE 13.—*Measurements of surface area of steers after fast*

Steer No.	Measured area of hide	Computed by Moulton's formula	Computed by Hogan's formula
	Square meters	*Square meters*	*Square meters*
57	4.774	4.542	4.131
60	4.717	4.429	4.324

TABLE 14.—*Derivation of values for surface area and maintenance requirement of net energy during the maintenance period preceding fast*

Steer No.	Surface area during fast	Fasting katabolism[a]		Empty weight of fasting animals	Loss of body tissue after maintenance until end of fast	Computed empty weight in maintenance period	Computed surface area in maintenance period	Maintenance requirement of net energy per head
		Total	Per square meter of body surface					
	Sq. meters	*Calories*	*Calories*	*Kilograms*	*Kilograms*	*Kilograms*	*Sq. meters*	*Calories*
57	4.774	7,838	1,642	363.4	15.4	378.8	4.809	8,044
60	4.717	7,034	1,491	333.1	14.4	347.5	4.843	7,221

[a] Fourth day of fast.

TABLE 15.—*Heat production, corrected for differences in live weight*

Steer No. and plane of nutrition	Period No.	Average live weight	Correction of heat production to basis of live weight of fast	Heat production			
				Uncorrected for live weight		Corrected to uniform live weight	
				Observed	Computed	Observed	Computed
Steer 60:		*Kilograms*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
Fast	15	383.0	0				
One-half maintenance	11	381.0	+25	7,572	7,564	7,597	7,589
Maintenance	1	310.9	+913	7,337	7,227	8,250	8,140
One and a half times maintenance	3	332.9	+628	8,963	8,781	9,591	9,409
Two times maintenance	5	358.4	+304	11,324	11,143	11,628	11,447
Two and a half times maintenance	7	391.8	−107	14,119	14,027	14,012	13,920
Three times maintenance	9	426.6	−524		16,224		15,700
Maintenance (alfalfa)	13		−187	9,916	9,616	9,729	9,429
Steer 57:							
Fast	14	399.2	0				
One-half maintenance	10	398.3	+12	8,047	8,050	8,059	8,062
Maintenance	2	359.6	+527	8,103	8,038	8,630	8,565
One and a half times maintenance	4	384.3	+196	9,791	9,591	9,987	9,787
Two times maintenance	6	403.1	−51	12,252	12,280	12,201	12,229
Two and a half times maintenance	8	443.7	−572	14,678	14,542	14,106	13,970
Maintenance (alfalfa)	12		−206	10,313	10,046	10,107	9,840

EXPERIMENTAL DATA

The foundation data of the experiments and the computations necessary for their interpretation comprise Tables 2 to 15, inclusive, while the derived final results are to be found in Tables 16 to 22, inclusive. For explanation of the significance of the experimental records and of the methods of computation employed (except for net-energy values) the earlier paper (4) on the subject of the present

discussion should be consulted. The treatment is practically identical in both papers (with the above-mentioned exception), and the arrangement of tables is the same. In the interest of economy of space, therefore, Tables 2 to 15, inclusive, are submitted for permanent record only, without discussion other than the following notes on unusual conditions. A change of attitude in regard to methods of computation of net energy is expressed in the discussion of Table 19, and Figures 3 and 4.

In period 1, with steer No. 60, and period 8 with steer No. 57, the data for the second day's metabolism were vitiated by technical errors. (Table 3.) The values used for the heat production in these periods, therefore, are the averages of the first and the third days' measurements.

It will be observed in Table 7 that the factor expressing the difference in the energy cost of standing as compared with lying is only one-third as large for steer No. 60 as for steer No. 57. No observations were made which explain this difference; but the higher value obtained with steer No. 57 raises a question as to whether this animal was more restless than steer No. 60 while standing, or whether steer No. 60 may not have leaned on the side of the stall for support while standing, during the fasting period, from the results of which this factor was derived. Whatever the significance of these data there was no recourse other than to use them as obtained.

The heat production of fast, as the base value in energy metabolism, enters into nearly all of the computations relating to the utilization of food energy. A change from the previous procedure was made in the treatment of the steers preparatory to the measurement of this value, in that grain alone was given during the last seven days of feeding, the idea being that the withholding of roughage during this interval would lead to a more complete evacuation of the alimentary tract and would make shorter the time required to reach complete fast.

The use of a physic in the preparation of the steers for the measurement of the heat production of fast was the same as in the previous year's work. that is, two doses were given, separated by a 2-hour interval, on each of the two mornings following the last allowance of feed.

In the previous year's study, in which the ration given in the interval immediately preceding fast was hay alone, the dry matter of the contents of the alimentary tract was reduced to 1.737 and 1.127 kgm., after seven and six days' fast, with steers Nos. 36 and 47, respectively; while in the experiments under discussion (Table 12), in which grain alone was given during the last seven days of feeding, the dry matter of the contents of the alimentary tract was reduced, after seven days' fast, to 0.527 and 0.335 kgm., with steers Nos. 57 and 60, respectively. The purpose of this change of procedure, therefore, was accomplished, in that the contents of the alimentary tract—already virtually negligible—were still further reduced.

In determining the heat production of fast it has been the custom at this institute to use for this measure the directly observed heat production, checked by the heat production as computed by the respiratory-quotient method. In the present series of experiments, however, neither of these measurements was entirely satisfactory. The direct measurement was affected by two unusual conditions in the

operation of the calorimeter which seem to have resulted in irregular flow of the cooling water and in unsatisfactory heat absorption. The calorimeter, therefore, appears not to have been in perfect balance during the fasting periods. The respiratory quotients obtained were improperly variable, apparently as a result of storing the air samples wet, and were not in harmony with the prevailing state of fast. Both of the measurements of heat production, therefore, seemed to be unreliable. Fortunately, however, the carbon dioxide measurements appear to have been correct, as shown by the fact that the gravimetric determinations were, on an average, 99.8 and 99.9 per cent of the Sondén determinations, with the two steers. The determinations by the two methods were made on separate aliquots of the air, the gravimetric determination in a continuous sample taken by a motor-driven pump, and the Sondén estimation on a series of intermittent air samples taken at intervals of 15 minutes.

In the light of later results—as yet unpublished—and in consideration of the method of preparation of the steers for the fasting experiments, it was assumed, with confidence, that these animals had reached a status of true fast, with a nonprotein respiratory quotient of approximately 0.707, by the beginning of the fourth day of fast—the first day of the fasting heat measurement—and on this basis the heat production of fast was computed.

The employment of this value as the heat production of fast completes the curves of heat production in relation to the plane of nutrition in a manner harmonious with the curves derived in the previous study, and therefore involves the same conception as to the significance of the fasting heat production, namely, that it comprises two factors, a waste heat of utilization of body nutrients katabolized, and a hypothetical minimum base value of the energy metabolism, including no such energy loss or expense of utilization.

In this light the use of the actual heat production of fast as the minimum base value of energy metabolism would appear to be justified only by the fact that it is measurable, while there is as yet no method known for estimating the lower, hypothetical, minimum value with accuracy.

The problem of standardization of the determination of the fasting heat production as the minimum, measurable base value of energy metabolism is being studied as the major item in the current program of experimentation at this institute.

In Table 8 is given the basis for the computation of the energy equivalent of the nitrogen eliminated per 12-hour subperiod, to be used, as shown in Table 10, in the computation of the total heat production of fast.

In view of the fact that the methods employed in these experiments did not make possible the determination of exact end points in the urinary nitrogen elimination there may be an appreciable error in the quantity given for energy of protein katabolized, and, therefore, a resulting error in the total heat production. A device has since been perfected for giving these desired end points with a high degree of exactness.

In Table 13 are given the measured areas of the removed hides of the experimental subjects, and for comparison the surface areas as computed by the methods of Moulton (*6*) and of Hogan (*5*). If the direct measurement of the removed hide is considered to be a correct

measure of the surface area, neither of the computation methods referred to gives nearly enough the same results to be satisfactory for this purpose. It is true that there is opportunity for error in even the direct measurement of the area of the removed hide, but the writers believe that this is the most nearly accurate measure of any thus far proposed.

In Table 15 are given the values of the directly observed and the computed heat production corrected for differences in live weight in order that they may be on a comparable basis. It will be noted that these corrections are in some cases extensive; thus in five experimental periods it was over 500 Calories in extent and varied between a plus correction of 913 Calories and a minus correction of 572 Calories. It is unfortunate that such extensive corrections of the heat production as observed, were necessary.

HEAT PRODUCTION IN RELATION TO THE PLANE OF NUTRITION

The observed and the computed heat production, corrected as in Table 15, are averaged and presented in Table 16, and are graphically shown in Figure 1 plotted against the dry matter of the feed eaten. (Table 2, or 5, or 17.)

TABLE 16.—*Average of observed and computed heat production, corrected for differences in live weight, and heat increments derived by comparison of the heat production in the periods of feeding with that of fast*

Steer No. and period	Plane of nutrition	Heat production	Fasting katabolism	Heat increments	
				Total	Per kilogram of dry matter
Steer 60:		*Calories*	*Calories*	*Calories*	*Calories*
Period 11	One-half maintenance	7,593	7,034	559	333
Period 1	Maintenance	8,195	7,034	1,161	411
Period 3	One and a half times maintenance	9,500	7,034	2,466	582
Period 5	Two times maintenance	11,538	7,034	4,504	790
Period 7	Two and a half times maintenence	13,966	7,034	6,932	922
Period 9	Three times maintenance	15,700	7,034	8,666	913
Period 13	Maintenance (alfalfa hay only)	9,579	7,034	2,545	508
Steer 57:					
Period 10	One-half maintenance	8,061	7,838	223	131
Period 2	Maintenance	8,598	7,838	760	246
Period 4	One and a half times maintenance	9,887	7,838	2,049	444
Period 6	Two times maintenance	12,215	7,838	4,377	702
Period 8	Two and a half times maintenance	14,038	7,838	6,200	770
Period 12	Maintenance (alfalfa hay only)	9,974	7,838	2,136	414

TABLE 17.—*Partition of energy of feed*

Steer No. and period	Dry matter of feed mixture	Energy per kilogram of dry matter							
		Gross	Digestible	Metabolizable	Total net	Feces	Heat increment	Methane	Urine
Steer 60:	*Kgm.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Period 11	1.681	4,468	3,202	2,540	2,207	1,266	333	439	222
Period 1	2.828	4,466	3,242	2,668	2,257	1,224	411	375	190
Period 3	4.237	4,468	3,178	2,653	2,071	1,290	582	350	175
Period 5	5.704	4,468	3,156	2,613	1,823	1,312	790	366	178
Period 7	7.520	4,468	3,094	2,589	1,667	1,375	922	335	170
Period 9	9.489	4,471	3,000	2,518	1,605	1,471	913	324	158
Steer 57:									
Period 10	1.700	4,468	3,249	2,584	2,453	1,219	131	438	227
Period 2	3.085	4,466	3,286	2,672	2,426	1,180	246	412	202
Period 4	4.612	4,468	3,279	2,704	2,260	1,189	444	386	190
Period 6	6.233	4,468	3,180	2,625	1,923	1,288	702	375	180
Period 8	8.057	4,471	3,121	2,588	1,818	1,350	770	364	169

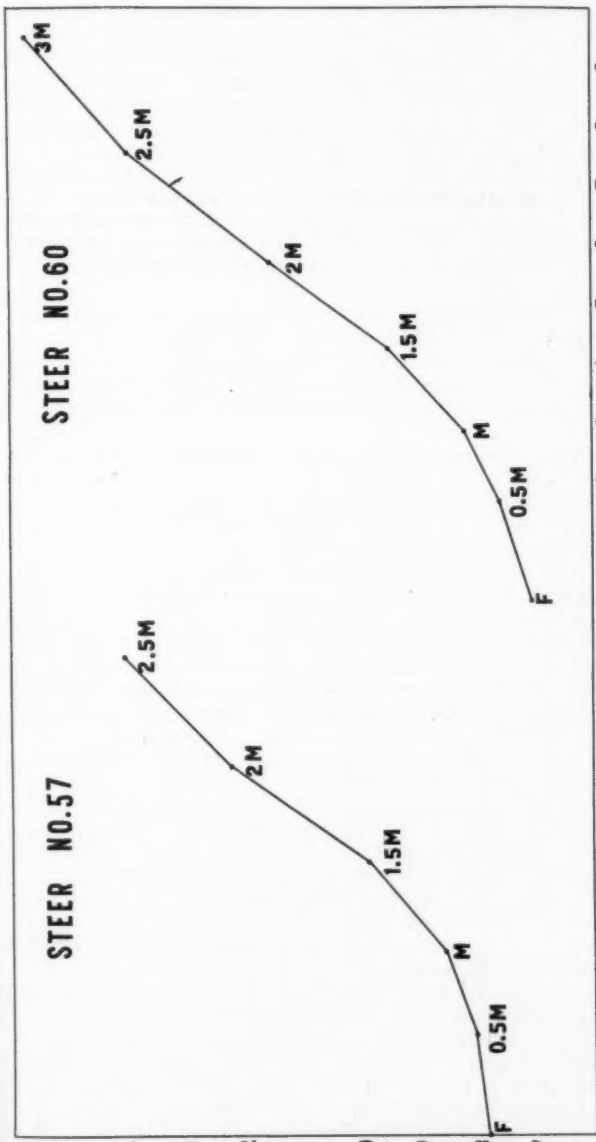

FIGURE 1.—Heat production as affected by the plane of nutrition of cattle

The curves representing this relationship confirm all details revealed in the earlier study of this subject; and add to the earlier presentation by extending the feeding with steer No. 57 to the plane of two and one-half times the maintenance requirement, and with steer No. 60 to three times maintenance—these extensions of the evidence continuing the tendencies manifest in the curves representing the earlier study.

This confirmation of the earlier findings is made especially impressive by the fact that the sequence of the experimental treatments (Table 1) was quite different from that in the earlier study, thus showing that the apparent relationship of the heat production to the plane of nutrition is real, and that the character of the curves representing this relationship is not determined, to any large extent, by a carrying over of an influence from one period to that which follows in the experimental program.

The curves representing the two animals are of identical significance. With the heat production of the fourth day of fast as the base value, the heat production increased slowly between fast and maintenance, and much more rapidly above maintenance, but with a decreased rate of rise between the planes of two and three times the maintenance requirement.

With largely the same understanding as expressed in the earlier paper (*4*), and with the conception as to the composition of the heat production of fast given on page 51, the curvature of the line of heat production in relation to increasing food consumption is interpreted as resulting from (1) the increasing concentration of metabolites circulating in the blood; (2) the change in the proportions of protein, fat, and carbohydrate katabolized, with increase in the katabolism of food nutrients and decrease in the katabolism of body nutrients; (3) the energy expense of synthesis of body nutrients (fat from carbohydrates); and (4) the decreased metabolizability of the food at the higher planes of nutrition—on account of which the quantities of food available for the use of the animal are not quite as great, proportionately, as the quantities eaten.

This assumes a difference in the energy expense of utilization of protein, carbohydrate, and fat in the metabolism of cattle. This assumption is made on the strength of the well-known difference in the specific dynamic effects of the three general classes of nutrients with other species; but with the understanding that on account of the heat of fermentation of carbohydrates, at least, if for no other reason, the relation of the energy expense of utilization of protein, carbohydrate, and fat in the metabolism of cattle must be different from that which characterizes the metabolism of the nonruminants in which this factor has been investigated.

The most interesting part of the curve of heat production is that between fast and maintenance, which expresses the resultant of the influence of body nutrients and of food nutrients metabolized.

A difficult conception necessary to the appreciation of the significance of this curve is that of the theoretical, minimum, base value of energy metabolism (referred to on p. 53)—the energy requirement of the animal living entirely on its own tissues, at 100 per cent efficiency of transformation of their metabolizable energy into vital energy; the heat production representing the actual energy need; there being complete oxidation of metabolizable nutrients, with no waste of heat in transformation, or through stimulation by metabolites; the organ-

ism existing, therefore, in a manner comparable to that of an internal-combustion engine operating under such hypothetical conditions that the work done would be equivalent to the potential energy of the fuel.

The difference between this minimum energy requirement of life and the heat production of the immobile animal during fast is the assumed energy expense, or waste, of utilization of the energy of the body tissue katabolized.

It is the magnitude of this observed heat production of fast which determines the lowest point, or base value, in this curve of heat production, and merely by so doing contributes largely to the determination of this curve between fast and maintenance.

For purposes of study and comparison similar curves of heat production as related to the metabolizable energy instead of the dry matter of the rations were drawn. These, however, are not presented, since, in view of the facts that energy is expended in the handling of the nonmetabolizable portion of the food, and that there is a closer relation between the dry matter and the heat production than between the metabolizable energy and the heat production, the dry matter is in reality a more significant base of reference than is the metabolizable energy.

This statement is based in part on the coefficients of correlation for the values entering into Figure 4. Thus the coefficients of correlation of the metabolizable energy and the heat production of mixed rations is 0.732 ± 0.026, and of the roughages is 0.76 ± 0.039; while the corresponding values for dry matter and heat production are, for mixed rations, 0.905 ± 0.011, and for roughages, 0.863 ± 0.015.

The curve of heat production in relation to the metabolizable energy is of the same character as the curve of heat in relation to the dry matter, though the degrees of curvature are somewhat less.

The decreased rate of rise in heat production at the highest planes of nutrition, which is to be seen in the curves based on the dry matter, is also present, though in reduced degree, in the curves based on the metabolizable energy.

ENERGY EXPENDITURE OF FOOD UTILIZATION

The net energy of a ration is the gross energy minus the energy of the excreta—urine, feces, and methane (which can be determined directly) and the energy expense of food utilization (the heat increment) which must be determined as the difference in heat production, as observed in two experimental periods, related to the concurrent difference in food.

In consideration of the fact that the quantitative relationship of the heat production to the food consumption at different planes of nutrition is such as to be expressed not by a straight line but by a reversed or S curve, it is obvious that the heat increase per unit of food, that is, the heat-increment value of the food, must differ with the plane of nutrition; and that the influence of the heat increment in the determination of net-energy values is such as to lead to a different net-energy value at each different plane of nutrition, though this influence is modified by the other factors which enter into the determination of net energy. Moreover, the conceptions of the significance and composition of the heat production of fast, as set forth on page 53, and of the factors which determine the curve of heat production in relation to

feed consumption, as stated on page 56, imply differences in the significance of heat increments above and below maintenance, especially in that heat increments between planes of nutrition below maintenance can not be considered as expressing only the energy expense of food utilization, as do heat increments above maintenance, but may be regarded as affected by the factor of waste heat of utilization of body nutrients.

The problem presented by the heat increment in relation to the determination of net-energy values is as to how these values shall be derived and how they shall be treated in order that they may contribute to the determination of net-energy values of the greatest practical usefulness.

In the light of the foregoing observations it will be understood that in a literal sense a net-energy value is exactly true only at the plane of nutrition at which it has been determined; but since it would be a hopeless task to derive such a series of sliding scales of net-energy values as is suggested by this fact, some arbitrary simplification must be adopted if the net-energy conception is to lead to any practicable system of measures in nutrition. With this desideratum in mind, therefore, the heat increments derived from these experiments will be considered.

The heat-increment values of the rations fed, based on the heat production of fast, are given in Table 16 arranged in order of rising planes of nutrition. All of these increments except the last, with each steer, apply to the mixed ration of hay and grain; the last applies to the ration of alfalfa hay alone.

If the heat production were a rectilinear function of the food energy it is obvious that the heat increments of the mixed ration, at the several planes of nutrition, however computed, would be alike; but in harmony with the observed relationship of the heat production to the plane of nutrition (Table 16 and fig. 1) the heat increments of the mixed ration, as computed with reference to the heat production of fast (Table 16) are characterized by such marked individuality that they could not consistently be averaged for any purpose. The increment between fast and maintenance, however, has a special significance as the energy expense of utilization of food for the preservation of energy equilibrium.

TABLE 18.—*Net-energy values for maintenance*

Period No.	Steer No.	Dry matter of feed eaten			Total heat production	Fasting katabolism	Heat increments		Metabolizable energy per kilogram of dry matter	Net energy per kilogram of dry matter (for maintenance)	Utilization of metabolizable energy (for maintenance)
		Alfalfa hay	Corn meal	Total			Total	Per kilogram of dry matter			
		Kgm.	*Kgm.*	*Kgm.*	*Cals.*[a]	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Per cent*
13	60	5.013	-------	5.013	9,579	7,034	2,545	508	1,927	1,419	73.6
12	57	5.155	-------	5.155	9,974	7,838	2,136	414	1,976	1,562	79.0
1	60	1.420	1.408	2.828	8,195	7,034	1,161	411	2,668	2,257	84.6
2	57	1.546	1.539	3.085	8,598	7,838	760	246	2,672	2,426	90.8
Computed values for corn meal.											
Do	60	-------	-------	-------	-------	-------	-------	313	3,415	3,102	90.8
	57	-------	-------	-------	-------	-------	-------	78	3,371	3,293	97.7

[a] Average of the observed and computed heat production corrected for difference in live weight.

TABLE 19.—*Net-energy values for body increase, the values for the individual feeds being computed by the heat-increment-proportional method, and by the net-proportional method*

Steer No.	Periods compared	Ration	Planes of nutrition compared	Metabolizable energy per kilogram of dry matter	Values by heat-increment-proportional method			Values by net proportional method		
					Heat increment per kilogram of dry matter	Net energy per kilogram of dry matter	Utilization of metabolizable energy	Heat increment per kilogram of dry matter	Net energy per kilogram of dry matter	Utilization of metabolizable energy
				Cals.	*Cals.*	*Cals.*	*Per cent*	*Cals.*	*Cals.*	*Per cent*
60	1 and 3	Hay and meal	Maintenance and one and one-half times maintenance	[a] 2,653	926	1,727	65.1	926	1,727	65.1
57	2 and 4	do	do	[a] 2,704	844	1,860	68.8	844	1,860	68.8
60	1 and 5	do	Maintenance and two times maintenance	[a] 2,613	1,162	1,451	55.5	1,162	1,451	55.5
57	2 and 6	do	do	[a] 2,625	1,149	1,476	56.2	1,149	1,476	56.2
60	1 and 7	do	Maintenance and two and one-half times maintenance	[a] 2,589	1,230	1,359	52.5	1,230	1,359	52.5
57	2 and 8	do	do	[a] 2,588	1,094	1,494	57.7	1,094	1,494	57.7
60	1 and 9	do	Maintenance and three times maintenance	[a] 2,518	1,127	1,391	55.2	1,127	1,391	55.2
60	1 and 3	Alfalfa hay	Maintenance and one and one-half times maintenance	1,916	1,145	771	40.2	830	1,086	56.7
57	2 and 4	do	do	2,000	1,420	580	29.0	802	1,198	59.9
60	1 and 5	do	Maintenance and two times maintenance	1,887	1,436	451	23.9	975	912	48.3
57	2 and 6	do	do	1,941	1,934	7	0.4	991	950	48.9
60	1 and 7	do	Maintenance and two and one-half times maintenance	1,870	1,520	350	18.7	1,016	854	45.7
57	2 and 8	do	do	1,914	1,841	73	3.8	952	962	50.3
60	1 and 9	do	Maintenance and three times maintenance	1,819	1,393	426	23.4	945	874	48.0
60	1 and 3	Corn meal	Maintenance and one and one-half times maintenance	3,396	705	2,691	79.2	1,023	2,373	69.9
57	2 and 4	do	do	3,411	268	3,143	92.1	885	2,526	74.1
60	1 and 5	do	Maintenance and two times maintenance	3,345	885	2,460	73.5	1,350	1,995	59.6
57	2 and 6	do	do	3,312	364	2,948	89.0	1,310	2,002	60.4
60	1 and 7	do	Maintenance and two and one-half times maintenance	3,314	937	2,377	71.7	1,447	1,867	56.3
57	2 and 8	do	do	3,265	347	2,918	89.4	1,237	2,028	62.1
60	1 and 9	do	Maintenance and three times maintenance	3,223	858	2,365	73.4	1,312	1,911	59.3

[a] Derived from supermaintenance periods (nos. 3–9) only.

A comparison of these heat increments, computed with reference to fast, with increments based on the heat production of maintenance, as in Table 19, shows that the latter are the higher values; thus, the heat-increment values between maintenance and two times maintenance are 1,149 and 1,162 Calories, while the increments between fast and two times maintenance are 702 and 790 Calories, respectively, for the two steers.

The difference in the heat increments computed on these two bases calls attention to the fact that it is the magnitude of the heat production of fast which is mainly responsible for the low heat-increment and high net-energy values for maintenance.

In the study of the supermaintenance heat increments, those between maintenance and planes of nutrition supplying multiples of the maintenance requirement seem to be convenient units in which to express and to consider the energy expense of utilization of food for body increase.

In Table 20 are presented the heat increments computed from the heat production of consecutive periods of nutrition. This method of computation ordinarily results in the maximum diversity of heat increments, since the differences in food are smaller than when each period is compared with one base value, while the inevitable element of error in heat measurements applies, in any case, to two such evaluations.

TABLE 20.—*Heat increments as computed from differences in the heat production of consecutive planes of nutrition*

Steer No.	Periods compared	Planes of nutrition compared	Heat increments per kilogram of dry matter
			Calories
60	15 and 11	Fast and one-half maintenance	333
57	14 and 10	do	131
60	11 and 1	One-half maintenance and maintenance	525
57	10 and 2	do	388
60	1 and 3	Maintenance and one and one-half times maintenance	926
57	2 and 4	do	844
60	3 and 5	One and one-half maintenance and two times maintenance	1,389
57	4 and 6	do	1,436
60	5 and 7	Two times maintenance and two and one-half times maintenance	1,337
57	6 and 8	do	999
60	7 and 9	Two and one-half times maintenance and three times maintenance	881

A comparison of these heat increments with those computed with reference to fast shows that the former are the higher values in all cases, except between the lowest two planes of nutrition, where the two methods of computation become one and the same.

The increments between fast and half maintenance are very low. With further rise in the plane of nutrition the heat increments increase rapidly to several times the values between fast and half maintenance; but, when computed from consecutive planes of nutrition, the increments at planes of nutrition above two times maintenance are lower than the maximum.

Obviously the influence of these extremely diverse heat increments on the derived net-energy values is prominent, but is modified by the values for the energy of the excreta—urine, feces, and methane—which also are involved.

A comparison of the heat increments for the two steers, as these values are computed by the three foregoing methods, shows that when based on the heat production of fast (Table 16) the heat increments for steer No. 57 are lower than those for steer No. 60, this difference appearing to be due especially to the high value for the fasting katabolism of steer No. 57. When based on the heat production of maintenance (Table 19), however, all of the increments of the mixed ration for steer No. 60 are the higher; as also are all but one of the increments as computed between consecutive periods. (Table 20.)

These comparisons suggest imperfect management of the calorimeter, or unsuccessful management of the animals with respect to treatment preparatory to fast, or different response of the animals to the fasting treatment, rather than that the energy expense of utilization of the food by steer No. 57 was lower than by steer No. 60, below the maintenance level of nutrition.

In the earlier study the corresponding heat increments for the mixed ration as determined with the two steers agreed very well; but the heat increments as determined in the present series of studies failed to agree, as representing the two animals, or in comparison with those determined the year before.

The study of these heat increments has not resulted in any entirely new conclusions, but has thrown new light upon the understanding derived from earlier studies, and has emphasized the importance of the following considerations:

(1) The necessity of rigidly standardizing the determination of the heat production of fast as the base value in the study of energy metabolism.

(2) The necessity of improving the basis for correcting the heat production, as observed, on account of differences in the live weight of the experimental subject in periods the heat production of which is to be compared. (See p. 39.)

(3) The necessity of determining separate net-energy values at the planes of nutrition of greatest significance, to wit, at maintenance, and at planes of nutrition at which food supplying multiples of the maintenance requirement of energy is fed.

PARTITION OF FEED ENERGY

The partition of the gross energy of the feed into its seven components—from the point of view of this study—is recorded in Table 17 and is graphically exhibited in Figure 2.

As in the previous year's work, the digestible energy increased slightly, with both animals, with increase of food from the half-maintenance to the maintenance level; and, viewing the two years' work together, it seems that this increase is due mainly to crude fiber, but partly to protein; and that ether extract and nitrogen-free extract do not contribute thereto.

With further increase in feed, from maintenance to the highest levels of nutrition, there was, with both steers, a slight but consistently continuous drop in digestible energy, this decrease, as in the previous year's work, being due mainly to nitrogen-free extract and protein.

The increased apparent digestibility of the protein of the ration from the half-maintenance to the maintenance level may be merely

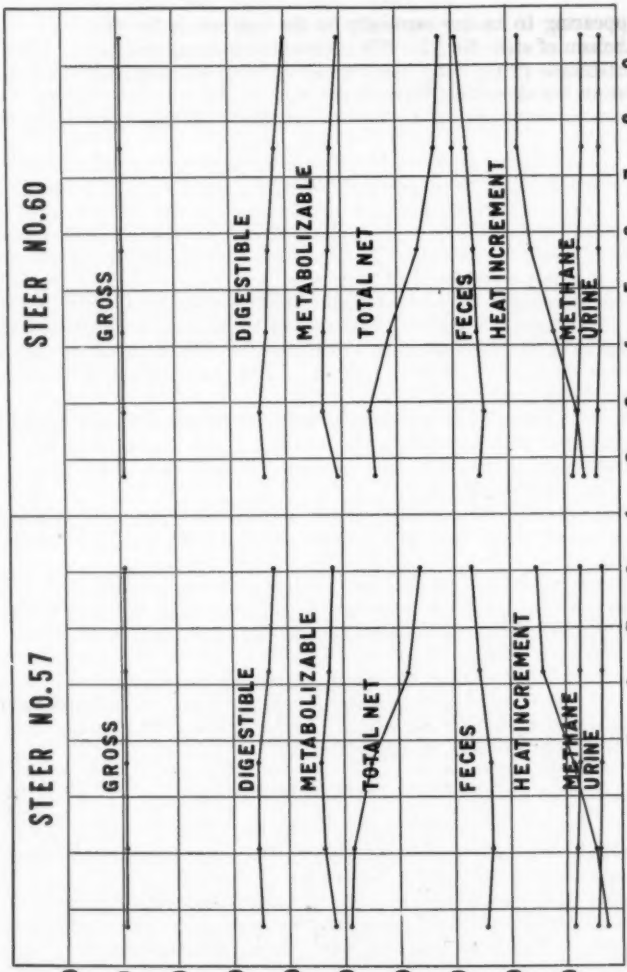

FIGURE 2.—Partition of feed energy as influenced by the plane of nutrition of cattle

an arithmetical result of a decrease in the proportion of metabolic to total protein in the feces; and the coincident increase in the digestibility of crude fiber may be a result of a decrease in some product of methane fermentation which (product) has the effect of limiting the digestibility of crude fiber at the half-maintenance as compared with the maintenance level—the methane fermentation having been found to be less, per unit of feed, during maintenance than during half-maintenance feeding; or the appearance of increased digestibility of both protein and crude fiber at maintenance as compared with half-maintenance planes of nutrition may possibly result merely from a carrying over of fecal residues from preceding high planes of feeding into the half-maintenance periods, though no direct evidence was observed in support of this suggestion.

The metabolizable energy, being the energy of the digestible nutriment minus that of the urine and the methane, follows closely the digestible, in its relation to the gross energy, but is an appreciably lower value than that representing digestible nutriment.

The metabolizable energy is almost exactly the same proportion of the gross energy at the highest and the lowest planes of nutrition, but at intermediate points is higher, the maximum being reached with one steer at the plane of half-maintenance, and with the other at half more than maintenance. The variation of the metabolizable energy in the previous study was closely similar.

The feces curve requires no comment, since it was the reverse of the curve of digestibility, which has been discussed.

The total heat increment (computed with reference to fast) increases with each rise in plane of nutrition, but with a decreasing rate of rise at the highest planes. From lowest to highest planes there is a prominent increase in the heat increment.

The urine and methane values are both small but appreciable in magnitude. They vary little throughout the several planes of nutrition; but decrease slightly, and with a close approach to regularity, from the lowest to the highest plane.

The total net energy—that is, the net for maintenance and body increase together—being the gross energy minus the energy of the urine, feces, methane, and heat increment, is expressed as a curve which is the resultant of the four curves representing the above-mentioned losses and expenses of food utilization.

From the lowest to the highest plane of nutrition there is a general and decided decrease in the net-energy value of the ration, at a rate approaching uniformity, but not being so nearly uniform as in the previous study.

In view of the fact that the primary purpose of net-energy values is to serve as convenient guides in feeding practice, it is obvious that the attainment of this objective requires the radical simplification of the truth, as expressed by these curves of total net energy, and that the method of accomplishing this simplification must be standardized in a conventional manner.

THE PROBLEM OF DETERMINATION OF NET-ENERGY VALUES OF RATIONS AND OF INDIVIDUAL FOODSTUFFS

It is axiomatic that the method of an investigation should reduce the problem to be solved to a single variant, a principle which, in relation to the present problem, involves the extremely exacting im-

plication that during a net-energy determination nutrients must be present of such kinds, quantities, and proportions as completely to satisfy all of the nutritive requirements of the animal for other than energy production, except as the nutrient reserves of the animal body protect it from deficiencies during the comparatively short interval of time covered by the determination; otherwise the energy metabolism must be affected.

With reference to this function the writers have to suggest only that, in the nature of the case, the detailed facts as to the length of time during which the nutritive reserves of the animal body protect it from the deficiencies of rations must vary, as determined by the status of these nutrient reserves, by the intimate character of the rations fed, and by the functional activities of the animal, in an exceedingly complicated manner.

In the routine practice of this institute the standard minimum time employed in a net-energy determination with cattle is 28 days—10 days in which to adjust the animal to the ration and 18 days for the determination of metabolizable energy. The last 3 of these 18 days are utilized also for the measurement of the heat production and respiratory products. It is, of course, impossible to say, in any particular case, how perfectly the nutritive reserves of the animal protect it, during this interval of 28 days, or during a series of such intervals, from shortage of nutrients in the rations fed.

As the net-energy conception antedates, in its origin, much of our present understanding of the details of nutrition, the recent advance in knowledge of the proteins, the vitamins, and the mineral nutrients has led to an understanding of the requirements of net-energy determinations as of continuously increasing complexity. While the principle of net energy is as simple and as irrefutable, therefore, as that of the bank balance, with which it is analogous, an actual net-energy determination really involves all of the intricacies of animal nutrition.

Prominent among the complications of this subject are those resulting from the relationship of the heat production to the plane of nutrition. Since this relationship is such as to be expressed by a reversed or S curve, its effect, as has been observed, is to determine that there are, fundamentally, as many net-energy values of a ration as there are points of observation as to the plane of nutrition—except as the above-mentioned curve of heat production happens to intersect a straight line representing a constant relationship of heat production to food consumed.

On this account the most practicable method of determination and use of net-energy values seems to be as representing definite intervals—as to planes of nutrition of special significance. For instance, a net-energy value determined at the level of energy equilibrium is highly significant, since it expresses the value of the ration for the definite purpose of maintenance; and such maintenance values possess a further significance in that they appear to be at least virtually the same as, if not quite identical with, net-energy values of feeds for milk production.

Naturally, the practical man has no interest in net-energy values applying to any plane of nutrition below maintenance.

Net-energy values for body increase may be determined at planes of nutrition arbitrarily fixed as requiring multiples of the main-

tenance requirement. The plane of twice maintenance seems to be especially significant in that it represents, in a general way, the average use of food, under conditions of practice. It is true that this is only a moderate rate of feeding; that full feed varies greatly with the character of the ration; that animals sometimes eat much more than twice maintenance; and that no one plane of nutrition is definitely representative of feeding for body increase. However, if determinations of net-energy values for body increase are to be made, as a standard procedure, at some one plane of nutrition—as the writers consider desirable—this must be at a plane at which the experimental animals can be depended on to eat up clean, without waste or refusal of parts, the more important and the greater number, at least, of the practical feeds; and to meet these requirements twice maintenance is provisionally adopted as a representative level of feeding for body increase. For special purposes, net-energy values might be determined at higher planes of nutrition.

The supplementing effects of foodstuffs on each other, as they are fed together, are also important in relation to the determination of net energy, since the net-energy values of nearly all feeds must be determined as the difference between results obtained with a particular ration and with the same plus the food product of interest. In such computations the entire supplementing effect of the combination is attributed to that component of the ration which is treated as the supplement, whereas, logically, it should in some way be apportioned between the basal portion of the ration and the supplement, as becomes obvious when a very small quantity of the supplement comes to be credited, by virtue of its apparent capacity to add to the effectiveness of the basal ration, with an absurd valuation.

The smaller the proportion of the supplement which is involved, the larger will be the unit effect of this influence. As an instance of this fact, it was shown by the senior author many years ago (*2*) in feeding experiments with growing swine, to which linseed meal and wheat middlings were fed in different proportions as supplements to corn, that the smaller the proportion of the supplement fed, and consequently the more deficient the ration in protein, the less efficient was the ration as a whole, as determined by the gain in weight in relation to feed eaten; but the greater was the supplementing, or corn-replacement value, of the protein supplements per unit of their own weight; and, we now understand, the greater must have been their net-energy values.

In the light of these facts it may be that there are, in reality, different net-energy values of feeding stuffs—for growing animals at least—in accord with each different nutritive ratio in which the feeding stuffs are combined. If such is the case, net-energy values should be determined in rations so compounded either that their digestible nutrients are contained in the optimum nutritive ratio, or that they represent especially significant conditions of practice.

It is also true that an accurate method has not yet been derived for computing the heat production, as observed in different experimental periods, and with the animal at different live weights, to the basis of the same live weight (see p. 39). Obviously it is important to improve the basis for this corrrection; and this is entirely practicable, but will require extensive studies, especially of the influence of

the plane of nutrition on the content of feeds and feed residues and on the heat of fermentation of carbohydrates in the alimentary tract.

Furthermore, there are serious arithmetical contributions to the difficulties of determining consistent net-energy values of foods. The most important of these derive from the fact that net energy can not be determined directly but is determined by difference—as a remainder, in the simplest case, after subtracting from the gross energy of of the food the energy represented by (1) the urine, (2) the feces, (3) the methane produced, and (4) the energy expense of food utilization—measured as the difference between the heat production at two planes of nutrition, related to the coincident difference in food—each of these four losses and expenses of utilization varying in a different way, as related to the plane of nutrition. The total of errors of work, whether additive or compensating, are obviously thrown into the remainder in the computation not only of the heat increment but also in the final computation of the net energy, as above outlined.

An idea of the mass of data affected by this principle may be derived from the fact that in a single 3-day balance of matter and energy, two of which are involved in the determination of one heat increment, as in the procedure outlined above, about 10,000 observations of one kind or another are made. It is true that the greater part of these observations are made in connection with the direct measurement of heat production, and that the number could be greatly reduced by determining the heat production by the respiratory-quotient procedure; but in the work of this institute, thus far, the direct measurement has been the more certain and accurate.

There is but one method, according to the procedures of this institute, for determining the net-energy value of a grain and of a roughage for maintenance. By this method the metabolizable energy of the roughage is determined directly from the feeding of a maintenance ration of roughage alone; the heat increment of the roughage is measured as the difference between the heat production of fast, and of maintenance on roughage alone; and the net energy is the difference between the metabolizable energy and the heat increment. Then the net-energy value of the grain is determined from the net-energy value of a mixed ration of grain and roughage (determined as above), by assuming the net-energy value of the roughage in this mixed ration to be the same as when the roughage is fed alone, the remainder being the net energy of the grain in the mixed ration.

This procedure has two weaknesses: The total effect of combining the grain and the roughage, and the resultant of all errors of work are assigned, by subtraction, to the grain alone.

Feed used for maintenance, however, is in an important sense a total loss, and the feeder's immediate interest is in the value of feeds for purposes of production after the animal's maintenance has been provided for. Very few individual feeds can be fed alone at practical rates of production; almost all feeds require to be fed as mixed rations; and one of the greatest difficulties in the application of the net-energy conception to the measurement of nutritive values of feeds is that of apportioning the net-energy value of a mixed ration—fed at a plane of production—between the components of such a ration, especially between the grain and the roughage.

For the attainment of this objective, that is, for the determination of separate net-energy values of a grain and a roughage, for body increase, three methods will be discussed (1) the feed-and-plane-difference method, (2) the net-proportional method, and (3) the heat-increment-proportional method. Each of these methods requires three experimental periods, one on a ration of roughage alone, at maintenance; a second on a ration of grain and roughage, at maintenance; and a third on a ration of grain and roughage, at the production level.

According to the feed-and-plane-difference method the net-energy value of the grain, for production, is determined by comparison of a ration of roughage, at maintanence, with a ration of grain and roughage, at the production level; while the net-energy value of the roughage, for production, is determined by a comparison of a ration of grain and roughage, at maintenance, with a ration of grain and a larger proportion of roughage, at the production level. This method involves, therefore, comparisons of rations differing not only in quantity but also in kind, and unproven assumptions (1) that the metabolizable-energy value of the roughage in the mixed ration at the production level is the same as that of the roughage when fed alone at the maintenance level, and (2) that the metabolizable-energy value of the grain is the same in the two mixed rations, of different composition, at the maintenance and the production levels.

In other words, this method involves any such errors as may result from the assignment—to whichever component (grain or roughage) is treated as the supplement— of the effects (on heat-increment value or metabolizable-energy value) of the difference in the planes of nutrition of the rations compared, and of the combination of the components of the mixed rations.

It is necessary, in order to minimize, as much as practicable, the supplementing effects just mentioned, to combine grain and roughage with due consideration of the proportions in which they would be fed in practice, especially in view of the fact that the smaller the proportion in which the supplement is fed, in the mixed ration, the greater will be the supplementing effect per unit of its own substance, and, consequently, the greater the distortion of its most significant net-energy value. Table 21 is presented as an illustration of this method of computation, the value derived in this case, on the basis of assumed data, being the net-energy value of grain for production.

TABLE 21.—*Example of the feed-and-plane-difference method*

Period No.	Dry matter of feed		Metabolizable energy	Heat production
	Roughage	Grain		
	Kilograms	*Kilograms*	*Calories*	*Calories*
1	5	2.5	18,000	12,625
2	5	None.	9,625	9,500
Difference equals	0	2.5	8,375	3,125
Difference per kilogram of grain equals			3,350	1,250

The net energy per kilogram dry matter of grain = 3,350–1,250 = 2,100 Calories.

According to the net-proportional method the determination of the separate net-energy values of a grain and a roughage for production

requires (1) a determination of the net-energy value of a mixed ration of the grain and the roughage, for production, by a comparison of such a ration at the production level with one of the same composition fed at the maintenance level, and (2) a computation of the net-energy values of the individual feeds, for production, based on the assumption that these values of the grain and the roughage for production are to their net-energy values for maintenance as the net-energy value of the mixed ration for production is to the net-energy value of the same for maintenance.

As an illustration of the net-proportional method the following is presented:

The net-energy value of the mixed ration of alfalfa hay and corn meal for maintenance as computed in Table 18, for steer No. 60, is 2,257 Calories per kilogram of dry matter. The net-energy value for body increase of the same mixture of alfalfa hay and corn, fed at a level of twice the maintenance requirement, as computed in Table 19, is 1,451 Calories per kilogram of dry matter. The ratio of net for body increase to net for maintenance is as 1,451:2,257, or as 0.643:1.

The net-energy values of alfalfa hay and of corn meal for maintenance of steer No. 60 are, as shown in Table 18, 1,419 Calories and 3,102 Calories per kilogram of dry matter, respectively. Hence, the individual net-energy values of alfalfa hay and of corn meal for body increase, at the two-times-maintenance level, are 912 ($1,419 \times 0.643$) Calories and 1,995 ($3,102 \times 0.643$) Calories per kilogram of dry matter, respectively. (Table 19.)

The fundamental assumption on which this method is based seems to the writers to be of doubtful validity, since it deals with the net energy as though it were of direct significance, whereas, as has been explained (p. 66), it is only a remainder, the character of which is determined as the difference between the gross energy of the feed, as the minuend, and the sum of the quotas of energy representing (1) the urine, (2) the feces, (3) the methane, and (4) the energy expense of food utilization, as the subtrahend. But whatever element of inconsistency there may be in the net-proportional method it seems unlikely that gross distortion of the true net energy values of individual feeds could result from the employment of this method since they are computed from the ratio of the net-energy value of a mixed ration for maintenance to the corresponding value for production, and since this ratio is not far from 1.

The heat-increment-proportional method requires the same number of experimental periods, with the same treatments, as are required by the net-proportional method—the computation of the individual net-energy values of grain and roughage for production depending on the assumption that their heat-increment and metabolizable-energy values for production are to their heat-increment and metabolizable-energy values for maintenance as the heat-increment and metabolizable-energy values of the combined ration of grain and roughage for production are to the heat-increment and metabolizable-energy values of the same for maintenance.

This assumption seems plausible, but is without basis of established fact and is characterized by serious practical shortcomings.

Thus, in computing the heat-increment value and the metabolizable-energy value of the grain and of the roughage for maintenance to

such values for body increase, the heat-increment values are multiplied by factors which may be several units in magnitude, while the metabolizable-energy values are multiplied by factors quite near to unity.

Such elements of error as there may be in the individual metabolizable-energy values of grain and of roughage, for maintenance, therefore, are not much altered in extent in the process of translation into values for body increase; but the errors in the heat-increment values of grain and of roughage, for maintenance, may be multiplied severalfold in being translated into values for body increase; and these exaggerated errors of heat-increment value may serve prominently to distort net-energy values, since they apply directly to the net, by subtraction.

Furthermore, since net-energy values of feeds for body increase are of lesser magnitude than are net-energy values for maintenance, the multiplied errors of translation of heat increment and metabolizable energy for maintenance to such values for body increase gain further power to distort net-energy values of feeds for body increase through this diminution of the base (net energy) affected.

Again, an error of any sort which affects the heat-increment or metabolizable-energy value of the mixed ration at either the maintenance or the production level thereby affects the factor for translation of maintenance values into production values, and so affects net-energy values for production.

As an illustration of the difference in the possibilities of distortion of heat-increment values and metabolizable-energy values, through multiplication of the element of error, by the net-proportional as compared with the heat-increment-proportional method, the following is presented:

In computing the heat-increment value of the mixed ration, for maintenance, in these experiments, to such a value at the two-times-maintenance level, by the heat-increment-proportional method, it is necessary (Tables 18 and 19) to multiply by the factors 2.83 (1,163÷411) and 4.67 (1,149÷246) for the two steers; while in the use of the net-proportional method the maintenance values of net energy are computed to production values at the two-times-maintenance level by the use of the factors 0.64 (1,451÷2,257) and 0.61 (1,476÷2,426) for the two steers.

As an example of the heat-increment-proportional method the following is presented.

The heat-increment value of the mixed ration of alfalfa hay and corn meal for maintenance of steer No. 60 is 411 Calories. (Table 18.) The heat-increment value of the mixed ration for body increase at the two-times-maintenance level is 1,162 Calories. The ratio of heat increment for body increase to heat increment for maintenance is as 1,162:411, or as 2.827:1.

The heat-increment values of alfalfa hay and of corn meal for maintenance of steer No. 60 are 508 Calories and 313 Calories, respectively. (Table 18.) Hence the heat-increment values of alfalfa hay and of corn meal for body increase, at the two-times-maintenance level, are 1,436 (508×2.827) Calories and 885 (313×2.827) Calories, respectively. (Table 19.)

The ratio of the metabolizable energy per kilogram of dry matter of the mixed ration at the level of two times maintenance to the metabo-

lizable-energy value of the same at the maintenance level (Tables 18 and 19) is as 2,613:2,668, or as 0.9794:1.

The metabolizable-energy values of alfalfa hay and of corn meal for steer No. 60 at the maintenance level are 1,927 Calories and 3,415 Calories, respectively. (Table 18.) Hence, the metabolizable-energy values of alfalfa hay and of corn meal at the two-times-maintenance level are 1,887 (1,927×0.9794) Calories, and 3,345 (3,415×0.9794) Calories, respectively. (Table 19.)

The net-energy values of alfalfa hay and of corn meal, for body increase at the two-times-maintenence level, are, therefore, 451 (1,887−1,436) Calories and 2,460 (3,345−885) Calories, respectively. (Table 19.)

The net-energy values which were determined in the present study will now be considered, those for maintenance being given in Table 18.

These figures for the mixed ration, for the alfalfa hay, and for the corn meal agree satisfactorily with the corresponding values as determined in the previous study—as they should, in view of the fact that nearly all controllable, contributing conditions were maintained the same in the two series of studies.

In Table 19 are given the net-energy values of the mixed ration, of the alfalfa hay, and of the corn meal at four supermaintenance planes of nutrition, the values for the mixed ration being computed by a direct, difference procedure, and for the individual feeds by the net-proportional and the heat-increment-proportional methods.

The heat-increment and the net-energy values of the mixed ration differ considerably among themselves at the several planes of nutrition, and show by their divergence from the results of the year before that the experimental conditions in the two years' work must have differed in some important regards. These may have been the length and the arrangement of the experimental program and the extensive change in the live weight of the subjects, which have been discussed.

It is not easy to reconcile the net-energy values of the alfalfa hay and of the corn, as derived by the heat-increment-proportional method, with those obtained for the same feeds, by the same method, in the previous study (*4*). Since the values of the mixed ration determined at two planes of production in the earlier study were virtually identical, and therefore satisfied practical interests, it has been natural to accept them as essentially correct; but if they are correct then the values determined in the present study are distinctly unsatisfactory, and the derived net-energy values of the individual feeds, when computed by the heat-increment-proportional method, are of such obviously erroneous character as to serve only to discredit the means by which they were derived.

During the progress of these experiments the general ensemble of attendant conditions (except in the fasting periods) was so unusually favorable that the work appeared to be virtually perfect, but the results, calculated by the heat-increment-proportional method, as applying to the individual feeds, are impossible.

Aside from the question of validity, the failure of these net-energy values to agree with the parallel data from the earlier study was probably due to the various complications and difficulties in the fundamental nutritional situation and in the necessary experimental procedure, which have been enumerated in the foregoing discussion; but especially to the fact (previously stated) that in using the heat-incre-

ment-proportional method any error in the determination of the heat increments for maintenance—as contributed, for instance, by the value employed for the heat production of fast—is multiplied in computing these maintenance increments to increments for production. Also there was the element of error (see p. 64) originating in the unusual length of the experimental program and due directly to the lack of an accurate method for computing the heat production from one live-weight basis to another.

This series of experiments, which was devised primarily to reveal the relation of the heat production to the plane of nutrition, and not for the purpose of net-energy determinations, seems not to have been well adapted to serve the latter purpose.

The net-energy values of the hay and of the grain as computed by the net-proportional method were more consistent than those which have been discussed. The values of both grain and hay at the plane of one and one-half times maintenance are appreciably higher than the values representing the higher planes; but the latter values for the hay and for the grain agree rather well.

TABLE 22.—*Average net-energy values of alfalfa hay, corn meal, and a mixed ration; obtained with four steers; the values of the individual feeds for body increase calculated by the net-proportional method*

Experiment and steer No.	Values when used for maintenance (net energy per kilogram of dry matter)			Values when used for body increase (net energy per kilogram of dry matter)		
	Alfalfa hay and corn meal 1:1	Alfalfa hay	Corn meal	Alfalfa hay and corn meal 1:1	Alfalfa hay	Corn meal
	Calories	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
238–47	2,241	1,385	3,111	1,557	963	2,162
238–36	2,218	1,395	3,059	1,541	969	2,125
240–60	2,257	1,419	3,102	1,482	932	2,037
240–57	2,426	1,562	3,293	1,610	1,037	2,185

In Table 22 are assembled the net-energy values of alfalfa hay, of corn meal, and of the mixed ration composed of these feeds from the present and the earlier experiment on the same subject. The values of the individual feeds for maintenance are computed by a direct, feed-difference procedure; and the values of the same at the several planes of nutrition above maintenance are computed by the net-proportional method and then averaged. These results seem promising, but they are few in number and their significance is not so thoroughly established as to warrant extended consideration.

In the previous study (*4*) the heat-increment-proportional method (*4, p. 294*) was preferred to the net-proportional method (*4, p. 293*) for computing net-energy values of single feeds for body increase, especially for the reason that in the use of the latter method the quantitative relation found to exist between the heat increments of a roughage and a concentrate for maintenance was reversed when this relation was computed as applying to feeding for body increase.

Such a reversal of relation of heat increments seemed inconsistent. Accordingly the heat-increment-proportional method was preferred, though the results obtained were recognized as peculiar and doubt was expressed as to the validity of the procedure. A somewhat different light is now thrown upon these two methods by a reexamination of the earlier work and by the results of the present study.

In comparing these two procedures, in the previous investigation (*4*), the heat increments were computed, as usual, on the basis of the dry matter of the feeds. It is now found, however (see the last columns of Tables 21 and 22 in the earlier paper and the last columns of Tables 18 and 19 of the present paper), that if the heat increments in either paper are compared on the basis of the metabolizable energy there is no such reversal as was observed of the quantitative relation of the heat increments of grain and hay at the different planes of nutrition.

In the present study there was an unusually good experimental basis for computing the heat-increment value of corn meal for body increase by the feed-and-plane-difference procedure, without the employment of either of the proportional methods. By comparing the heat production from a maintenance ration of alfalfa hay (period 13) with the heat production from a mixed ration consisting of about this same quantity of alfalfa hay plus an equal quantity of corn meal (period 9), it was found that the heat-increment value of the corn meal (1,280 Calories) was higher than that of the mixed ration of corn meal and alfalfa (1,127 Calories)—derived from a comparison

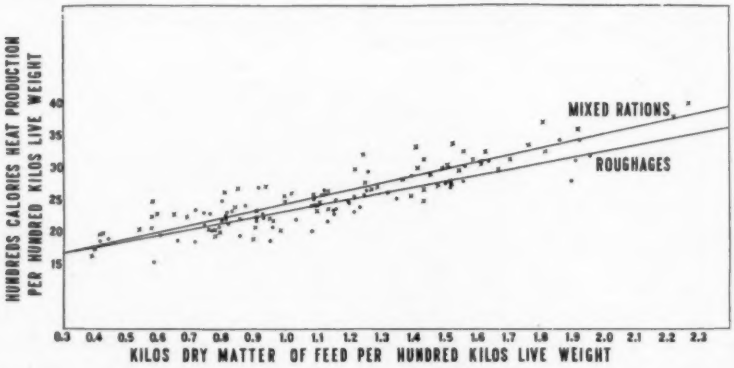
HUNDREDS CALORIES HEAT PRODUCTION PER HUNDRED KILOS LIVE WEIGHT
40
35
30
25
20
15
MIXED RATIONS
ROUGHAGES
0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2.0 2.1 2.2 2.3
KILOS DRY MATTER OF FEED PER HUNDRED KILOS LIVE WEIGHT

FIGURE 3.—The heat production of cattle as related to the dry matter of mixed rations and rations of roughage only

of the above-mentioned ration of corn and alfalfa (period 9) with another ration of the same composition fed at the maintenance level (period 1)—thus indicating that the heat-increment value of corn for body increase is actually higher than that of alfalfa hay—in harmony with the results of the earlier study (*4*) and of the present one as well, when computed by the net-proportional method.

In consideration of the fact that corn meal is considerably richer in metabolizable energy than is alfalfa hay it might be expected to have a higher heat-increment value if the relation were a direct and simple one. In this light the observations just cited seem consistent; but in the present as well as in the previous study the maintenance heat-increment value of corn meal is less than that of alfalfa hay—which directs attention to the fact, explained in the previous paper (*4, p. 254*), that the heat increment is a value of exceedingly complex origin; and in consideration of the number and diversity of its components, the above suggestion that these components, and the heat increments as a whole, do not vary, as affected by the plane of nutrition, in the simplest proportional manner, is not surprising.

Further light is thrown upon this matter by a statistical analysis, shown in Figure 3, of the results of published work of this institute, comparing the heat production from rations of roughage alone with that from mixed rations of grain and roughage.

Fifty-four experiments with roughage alone and 77 with grain and roughage are represented as two groups, by lines of regression representing the following equations for the relation between heat production and dry matter: For roughage rations $y=0.931\ x+1{,}386$; for mixed rations $y=1.060\ x+1{,}385$.

In viewing this graph the reader should bear in mind that the divergence of the line representing the mixed rations from that representing the roughage rations expresses only the influence of the grain in the mixed rations, and that a line representing grain alone would be much more divergent from that representing roughage. The graph shows that, on an average, the concentrates used in the mixed rations led to greater heat production than did the roughages—which tends to corroborate the net-proportional method, and suggests, therefore, that the assumption on which the heat-increment-proportional method is based, which assigns lower heat-increment values to the corn meal than to the alfalfa hay (Table 19), is incorrect.

Thus, the average heat-increment value per kilogram of dry matter of the mixed rations represented in this graph was 1,060 Calories, and of the rations of roughage alone, 931 Calories, and in the present study almost identical values were derived; that is, 1,076 Calories for the mixed ration and 930 Calories for the alfalfa hay, for body increase, when computed by the net-proportional method; while the heat-increment-proportional method assigned to alfalfa hay an average heat-increment value of 1,527 Calories. The heat-increment value and the corresponding net-energy value of the corn meal, as obtained by the direct comparison of data from periods 9 and 13 (p. 72), agree fairly well with the corresponding values computed by the net-proportional method. These values for heat increment were 1,280 Calories and 1,312 Calories, and for net energy, 1,835 Calories and 1,911 Calories, respectively, as computed by the two methods.

Also, the net-energy values obtained by the net-proportional method are in fair agreement with the values derived for the same feeds by Armsby (*1, p. 660*) and by two of the present writers (*3, p. 1098*).

In Table 23 are assembled heat-increment values of alfalfa hay determined directly, in supermaintenance periods in which the hay was fed by itself (experiments 212 and 216), for comparison with the heat-increment values of alfalfa hay, from the last two years' work, computed by the net-proportional and by the heat-increment-proportional methods.

The results computed by the net-proportional method agree fairly well with the directly determined values. The results computed by the heat-increment proportional method are much higher and more variable—higher, as a result of the difference in the assumptions on which the methods are based, and more variable because of the multiplication of experimental errors by the heat-increment-proportional computation.

TABLE 23.—*Comparison of the heat-increment values of alfalfa hay (1) determined directly and computed (2) by the net-proportional and (3) by the heat-increment-proportional methods*

Experiment No.	Periods compared	Heat-increment values determined—		
		Directly	By the net-proportional method	By the heat-increment-proportional method
		Calories	*Calories*	*Calories*
212 (3) 216 (3)	3 and 1	980		
	4 and 2	934		
	6 and 5	1,056		
	Average	990		
238 (4)	1 and 7		957	1,286
	2 and 8		984	1,342
	3 and 7		1,043	1,425
	4 and 8		1,036	1,425
[a] 240	1 and 3		830	1,145
	2 and 4		802	1,420
	1 and 5		975	1,436
	2 and 6		991	1,934
	1 and 7		1,016	1,520
	2 and 8		952	1,841
	1 and 9		945	1,393
	Average		957	1,470

[a] Results presented in present paper.

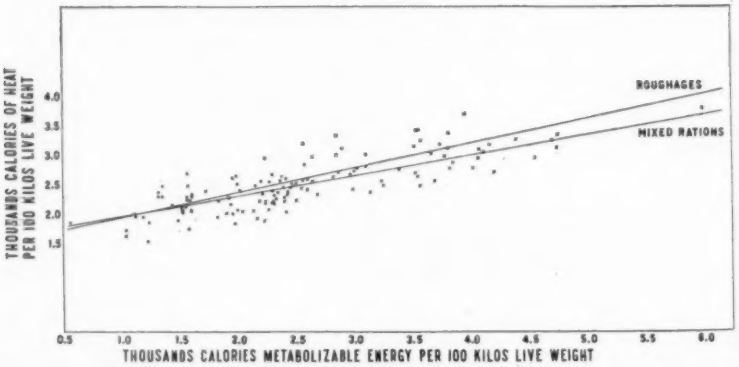

FIGURE 4.—The heat production of cattle as related to the metabolizable energy of mixed rations and rations of roughages only

In relation to the relative energy expenditure of utilization of grain and of roughage feeds, in general, it is significant (1) that the quantitative relationship of heat-increment values of grain and roughage shown in Figure 3 would be reversed if the heat production were related to the metabolizable energy instead of to the dry matter, as in Figure 4; (2) that the higher rate of increase in heat production from grains than from roughages—with increase in dry matter of feed consumed—is due in part to the higher metabolizable energy content of the grains (fig. 3); and (3) that the higher rate of increase in heat production per unit of metabolizable energy of roughage is presumably due in part each to the greater quantity of dry matter per unit of metabolizable energy, and to the more resistant physical character of the dry matter of such feeds. (Fig. 4.)

The following are the equations for the relation between the heat production and the metabolizable energy represented by the lines of regression of Figure 4: For roughage rations $y=0.419\ x+1{,}526$; for mixed rations $y=0.343\ x+1{,}615$.

It is the opinion of the writers that the evidence at hand does not afford a satisfactory basis for final choice between the foregoing methods of computation of the results of a net-energy determination; and they defer judgment on this matter until a considerable body of results shall have been obtained in experiments especially planned for the derivation of net-energy values, in the light of the present ideas of the authors as to essential requirements.

From the foregoing recountal of the difficulties and compromising conditions inherent in the determination of net-energy values it will be understood that these measures can not be considered as of absolute value; in fact the determination of net-energy values must be so rigidly and arbitrarily standardized and controlled that the results, in spite of the particularity of the method of determination, must be regarded as, to a considerable extent, conventional.

The net energy conception, however, expresses a simple, logical, and scientific point of view from which to regard the supremely important subject of energy metabolism; and a natural approach to the subject is through a study of the factors which affect the net energy. Also, no other measure of the energy values of foods has been proposed which has a scientific validity at all approaching that of net-energy, in spite of all the compromises involved.

An important problem in connection with the use of net-energy values is that of the terms in which they are to be stated; and the utmost practicable simplicity in the system is imperative.

Armsby, Kellner, and Møllgaard have all stated nutritive values based on the net-energy conception in terms of a single unit; that is, all three have stated the energy requirements for maintenance, for body increase, and for milk production in terms of values for body increase, though Armsby's production values were, by the method of determination, somewhat mixed with net energy for maintenance.

A somewhat different light is now thrown upon this problem—in the authors' minds, at least—by their studies on the heat production of fast; by their adoption of the directly determined heat production of fast as the base value in energy metabolism of cattle and as the measure of the net-energy requirement of maintenance; by their demonstration of the relation of the heat production to the plane of nutrition; and by the demonstration, by Møllgaard and themselves, of the essentially different net-energy value of a food for body increase, on the one hand, as compared with maintenance and milk production on the other.

Though a simple system of designation of nutritive values seems no less desirable, on account of this recent progress, it is now clear that to use a single net-energy value, as a measure of nutriment for maintenance, milk production, and body increase, would be to apply it to a much more complicated situation, as to economy of utilization of food energy, than was formerly understood.

The question above suggested is, fundamentally, whether the essential difference in the economy of utilization of food energy for maintenance and milk production, as compared with body increase, shall be expressed in terms of different net-energy values, or whether,

for the sake of convenience, one net-energy value is to be used, and the difference in economy of utilization of food energy is to be expressed in the feeding standard. The authors are inclined to reserve their decision on this point, pending the settlement of the more urgent, fundamental problems involved in the net-energy determination.

In the light of the foregoing discussion, therefore, it is proposed to adopt a standard arrangement of the experimental periods in a program of determinations of these measures. Thus, in order to derive the greatest profit from such experiments, with cattle, it is advantageous to determine the net-energy values of a grain and of a roughage, for maintenance and for body increase, in one experimental program; and the writers suggest that the schedule of treatments should be arranged as follows:

Treatment	Period
Roughage and grain; two times maintenance	1
Roughage and grain; maintenance	2
Roughage; maintenance	3
Fast	4
Slaughter; to determine surface area	5

This arrangement provides, as a standard procedure, that fast shall follow maintenance; and also that there shall be the minimum differences between the live weights of the animal in the periods which must be compared, thus requiring the minimum correction of the heat production, as observed, in computing to the basis of one live weight.

The above schedule of experimentation, however, is not suggested as one to be adhered to in an absolutely fixed manner. For special purposes it may be varied; but it seems to be well adapted, as it stands, to serve the most extensive of present requirements.

In the derivation of such a system of measures it is essential that the energy metabolism be referred to a definite base value; and a series of experiments has been conducted at this institute having for their object the definition—as such a base value, and as the measure of the maintenance requirement of net energy—of the conditions of determination of the heat production of fast.

Though this study is not yet finished the authors consider it desirable to make as complete a statement as possible at this time of their position with reference to the problem of determination of net-energy values. They therefore anticipate the full publication of this research by proposing the following statement of standard conditions for the determination of the heat production of fast, as the base value in energy metabolism, and as the measure of the maintenance quota of net energy: The heat production of the first day, beginning either in the morning or the evening, following the appearance, within the animal, of a status of metabolism characterized by the nonprotein respiratory quotient of fat—this measurement to follow a preparatory feeding at the plane of energy equilibrium.

SUMMARY

A series of 15 experiments was conducted, with 2-year-old Shorthorn steers as subjects, by direct heat measurements checked by indirect calorimetry, for the study of energy metabolism in relation to the plane of nutrition, especially as bearing on the problem of method of determination of net-energy values of feeding stuffs.

The energy metabolism was studied at seven planes of nutrition, namely, (1) fast, (2) half of the maintenance requirement, (3) maintenance, (4) half more than maintenance, (5) two times maintenance, (6) two and a half times maintenance, and (7) three times maintenance.

In the feeding periods the rations were composed of corn meal and alfalfa hay, in equal weights of dry substance, except in one period with each steer, in which the ration was alfalfa hay alone.

In the preparation of the steers for the fasting heat measurement grain alone was given during the last seven days' feeding. This treatment, together with the physicking of the steers, reduced the contents of the alimentary tract to negligible quantities.

With the heat production of the fourth day of fast as the base value, the heat production increased slowly between fast and maintenance, and much more rapidly above maintenance, but with a decreased rate of rise between the planes of twice and three times maintenance.

The curve of heat production in relation to the plane of nutrition was found, therefore, to be a reversed or **S** curve.

The heat production of fast being considered as including two factors, a waste heat of utilization of body nutrients katabolized, and a theoretical minimum base value, including no such waste—the curvature of the line of heat production in relation to increasing food consumption is interpreted as resulting from (1) the increasing concentration of metabolites circulating in the blood; (2) the change in the proportions of protein, fat, and carbohydrate katabolized, with increase in the katabolism of food nutrients and decrease in the katabolism of body nutrients; (3) the energy expense of synthesis of body nutrients (fat from carbohydrates); and (4) the decreased metabolizability of the food at the higher planes of nutrition.

The heat increments between the lower planes of nutrition were considered to be less than the true energy expense of food utilization by the amount of the waste heat of utilization of body nutrients katabolized.

Attention is called to the fact that on account of the extensive fermentation of carbohydrates, with accompanying heat production, in the ruminant alimentary tract, the relative energy expenses of utilization of protein, fat, and carbohydrates, as found in other animals, do not prevail in ruminants.

The authors conclude that the most practicable possibility of deriving a system of energy values of feeding stuffs, to serve as guides in practice, requires the continued adherence to the general point of view and method of Armsby, but modified, in the light of recent findings at this institute, (1) by the adoption of the standardized, directly observed heat production of fast as the base value in the energy metabolism of cattle, and as the measure of the maintenance requirement of net energy, and (2) by the recognition of the facts as to the relation of the energy metabolism to the plane of nutrition, which imply the existence of a fundamentally different net-energy value of a feeding stuff at each point of observation, in relation to the plane of nutrition, and which necessitate a practical recognition of the different rate of economy of utilization of food energy for body increase as compared with maintenance.

The authors suggest the following statement of standard conditions for the determination of the heat production of fasting cattle, as the

base value in energy metabolism, and as the measure of the maintenance quota of net energy: The heat production of the first day—beginning either in the morning or the evening—following the appearance, within the animal, of a status of metabolism characterized by the nonprotein respiratory quotient of fat; this measurement to follow a preparatory feeding at the plane of energy equilibrium.

The most difficult unsolved problem in net-energy determination is the separation of the net energy of grain from that of roughage (hay), since neither can be fed alone, at practical levels of production, and since combining the two has the effect of altering the rates of economy at which they are utilized.

Methods of computation of the results of net-energy determinations are critically discussed, and a standard schedule of experimentation for net-energy determinations is proposed.

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BIOLOGICAL VALUES AND SUPPLEMENTARY RELATIONS OF THE PROTEINS IN ALFALFA HAY AND IN CORN AND SUNFLOWER SILAGE[1]

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INTRODUCTION

The conception of the net-energy values of feeds, as worked out by Armsby (*1*),[3] renders accurate comparisons of feeds possible in respect to their energy content for various types of production and maintenance. Owing to the complexity of the protein molecule, in which quantitative and especially qualitative differences occur according to the source, no such direct method of comparison has been developed for the protein content. A molecule of a complete protein may consist of a chain of 30 (*14*) or more alpha amino acids, some of which are complex and others simple, some present in abundance, others only as traces, according to the nature of the protein.

The most accurate method of evaluating proteins available at the present time appears to be that employed by Thomas as modified by Mitchell (*6*). This method takes into consideration the body nitrogen in both the urine and feces during protein feeding as determined by feeding rations nearly free of nitrogen.[4] This method can be used with a single protein, as well as with protein mixtures. It is particularly valuable for determining in which feed combinations the protein mixtures are most effective in supporting production and maintenance.

It is a well-established fact that proteins deficient or low in one or more of the necessary amino acids can be rendered highly efficient through the proper combination with proteins rich in the missing acids.

PLAN OF THE EXPERIMENTS

The chief aim in this study was to determine the biological values of the proteins in alfalfa hay, sunflower silage, and corn silage when fed singly and in combination to lambs. Mixtures of 3 parts of silage to 1 of hay were selected, since these are so commonly used in practice

In studying any feed or feed combination, six lambs were fed for a 10-day preliminary and a 10-day experimental period. Nearly nitrogen-free rations were fed before and at the conclusion of the experimental series of rations. The order of feeding as actually planned and carried out was as follows: (1) Nearly nitrogen-free rations; (2) alfalfa hay; (3) sunflower silage; (4) 1 part alfalfa hay, 3 parts sunflower silage, by weight; (5) corn silage; (6) 1 part

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[2] Thanks are due the members of the Division of Chemistry, in whose laboratory the writer did the analytical work, and to H. Hackedorn, head of the Department of Animal Husbandry, for helpful suggestions.

[3] Reference is made by number (italic) to "Literature cited," p. 96.

[4] Protein in this discussion is considered to be nitrogen × 6.25.

alfalfa hay, 3 parts corn silage by weight; (7) nearly nitrogen-free rations.

The entire field work was completed in 140 days, during which a quantitative collection of feces and urine was made. However, between each 10-day period, at least 1 or 2 days were necessary for a complete cleaning of the crates before a new period was started.

There were three metabolism crates available for use, and while three lambs were being fed, the others were undergoing preliminary feeding, preparatory to the next period. In this manner the field and chemical work could go on simultaneously with little danger of losing samples through spoilage or deterioration.

CHEMICAL DETERMINATIONS

All nitrogen determinations were made by the official Kjeldahl-Gunning-Arnold method for organic nitrogen. Official methods of analysis were used throughout this study.

FACTORS WHICH AFFECT BIOLOGICAL VALUES

The nutritive value of the proteins in any single feed mixture is taken as the total nitrogen retained by the experimental animal in percentage of the total nitrogen absorbed. This percentage is called the biological value of the protein and measures the value of a protein in all the anabolic reactions of the body.

It is apparent that differences in digestibility are not involved in determining the biological value of proteins. However, the biological value of the protein is expected to vary (*7*) with the use to which the protein is put by the body. These differences are related to the amino acid make-up of the protein as compared with the several separate requirements for amino acids. Furthermore, under any one set of experimental conditions the biological value of a protein is fixed by its constitution.

The biological value is, then, (*5*) determined by the ratio of the percentages in food protein and body complex, respectively, of the amino acid for which this ration has the lowest value, unless the body has the capacity to synthesize that particular amino acid from others.

Two assumptions in such studies are ordinarily made: (1) That the undigested fraction of a protein is of the same amino acid make-up as that digested; (2) that in protein hydrolysis, amino acids split off from the molecule at equal rates, or else that tissues can store those splitting off first until others form, so that optimum proportions of amino acids are secured for body needs.

It is also assumed that no protein is catabolized for energy purposes until all requirements for body proteins are met, excluding, of course, that protein or its fraction not available for body needs.

Mitchell (*7*) has demonstrated for several proteins that an increase in the level of protein intake results in a lowering of the biological value of the protein. The lowering occurs as the concentration of the protein in the ration increases, but is not proportional to the quantity of protein intake, as influenced by variations in feed consumption.

During the feeding of nearly nitrogen-free rations for ideal results, no protein of the body should be used as a source of energy, except

those fractions of the protein molecule not completely utilized for maintenance and various forms of production.

The body's contribution of nitrogen to the urinary and fecal excretions during periods of protein feeding then is measured by the nitrogen content of the urine and feces of the experimental animal when receiving a near-nitrogen-free ration rich in net energy, the feed intake being such as to exceed liberally the energy maintenance requirement.

If the fecal nitrogen excretion on a nitrogen-free diet is to be taken as a measure of the excretion of metabolic fecal nitrogen during subsequent experimental periods in which rations containing protein are fed, it is advisable to equalize the fiber content as nearly as possible in both the nonnitrogenous and nitrogenous rations. Mitchell (*6*) points out that failure to equalize the fiber results in an underestimation of metabolic nitrogen and to an overestimation both of food nitrogen retained in the body and of the absorbed nitrogen, the numerator and denominator, respectively, of the fraction determining the biological value. For this reason this error is not considered very serious.

It has also been shown that the excretion of nitrogen in the feces of animals on diets containing minimal amounts of nitrogen (0.04 to 0.08 per cent) varies directly with the amount of food consumed.

The best method, then, of estimating metabolic nitrogen in the feces of animals on rations containing protein is to determine in a period of nonnitrogenous feeding the excretion of fecal nitrogen per gram of dry matter of the food consumed and to apply this figure to the amount of dry matter of the protein-containing food consumed in subsequent experimental periods.

An indirect way of measuring urinary nitrogen of endogenous origin is to eliminate entirely the exogenous catabolism by the feeding of a nitrogen-free diet, and inducing the animal to eat enough of the ration to provide for its energy requirements, thus preventing oxidation of body tissue with its accompanying loss of nitrogen, which can not properly be called endogenous nitrogen. The assumption is made that the catabolism of body substances containing nitrogen, occurring during feeding of nitrogen-free rations high in energy, continues at the at the same level during protein feeding.

The quantity of nitrogen in the urine per 100 gm. of body weight during periods of feeding nearly nitrogen-free rations, when applied to the weights of the same animals fed protein rations, is then the measure of the endogenous urinary nitrogen.

The nitrogen in the urine and feces does not completely account for the total loss of nitrogen from the body. To these should be added the loss through wool and epidermis. In sheep, however, this quantity is small, and is disregarded, especially in short-time experiments.

The biological value expressed in a mathematical form is as follows:

$$\text{Biological value} = 100\frac{[(\text{Food N}-\text{food N in feces})-(\text{Urine N}-\text{Body N in urine})]}{\text{Food N}-(\text{N in feces}-\text{body N in feces})}$$

or

$$\text{Biological value} = 100\frac{\text{Food N retained}}{\text{Absorbed N}}$$

Mitchell (*9*) has pointed out that the determination of the biological value is unaffected by the position of the experimental period in rela-

tion to the near-nitrogen period. Among 17 possible comparisons, 9 favored the view that low-nitrogen feeding increases the biological value in an immediately subsequent period, 6 were opposed to this view, and 2 comparisons showed no difference.

NITROGEN METABOLISM STUDIES

The lambs were fed in metabolism crates previously described (*13*). Every feed combination was studied with six lambs, each fed over a 10-day preliminary and a 10-day experimental period.

The urine samples were collected daily, and the largest portion of feces was also collected daily except at the end of each period, when the crates were cleaned and washed. The urine was kept in carboys and the feces in air-tight galvanized iron cans. The total urine and feces from each sheep were carefully sampled (*2*) and analyzed soon after sampling. All the sampling was done rapidly and at fairly low temperature, and the samples were immediately analyzed to minimize losses in moisture.

Body weights were taken at the beginning and at the end of each period.

The roughage feed for any particular trial was weighed out at one time and placed in paper sacks. A representative sample taken at this time for chemical analysis served as the basis for computing the nutrients fed.

Fresh silage was taken from the silo each morning, completely mixed, and the daily allowance for each lamb weighed out and placed in air-tight jars. Occasional handfuls of silage were placed in a sample jar for analysis. These daily samples were dried, and at the end of each 10-day experimental period a composite sample was prepared for chemical study.

Unconsumed feed was collected daily, air-dried, ground, and sampled. The nutrient intake was then computed by subtracting the nutrients in the refuse from those in the feed consumed. In one instance during the feeding of the near-nitrogen-free rations the total grams of nitrogen in the unconsumed feed exceeded the nitrogen in the feed consumed. A careful recheck led to the possible explanation that the saliva in the feed refused contributed the excess nitrogen.

A further description of methods is given in a previous paper (*13*)

NEARLY NITROGEN-FREE RATIONS AS A MEANS OF DETERMINING PROTEIN MAINTENANCE REQUIREMENTS

The fact is well established that when a ration containing net energy in excess of the maintenance requirement is fed the total nitrogen excreted in the urine and feces is less than during complete fasting. The feeding of nonnitrogenous nurtients tends to diminish the catabolism of proteins to the minimum. However, even a liberal supply of energy will not completely prevent the breaking down of tissues.

Robison (*12*) assumed that the minimum nitrogen requirement of maintenance is represented by the sum of the nitrogen in the urine and that in the feces during periods of feeding liberal nearly nitrogen-free rations.

Mitchell (*8*), however, points out that the maintenance requirement for nitrogen by any animal may be measured by the total daily excretion of nitrogen in the urine alone, to the exclusion of the fecal nitrogen.

Although the fecal nitrogen is of body origin, it varies with the dry matter consumed, and would vary with the type of maintenance ration used, increasing with a corresponding increase of the ration. It appears that the fecal nitrogen should be considered as a wastage to be subtracted from the feed nitrogen.

Table 1 contains the data of twelve 10-day metabolism experiments, during which nearly nitrogen-free rations consisting of linseed oil, cornstarch, cane sugar, and selected stems of Albit wheat straw were fed. The average of 0.664 pound of protein per 1,000 pounds live weight for lambs averaging 24.34 kgm., determined according to Robison (*12*), compares favorably with the requirements of Schulze and Märcker cited by Armsby (*1*). These investigators recommend 0.653 pound digestible crude protein daily per 1,000 pounds live weight for maintenance.

A study of the individual maintenance requirements shows a minimum of 0.495 and a maximum of 0.886 pound of digestible crude protein required daily for maintenance per 1,000 pounds live weight.

Data for the urinary nitrogen are also reported in Table 1, and from these figures the maintenance requirements may be computed according to the suggestion of Mitchell (*8*).

TABLE 1.—*Maintenance requirements of lambs averaging 24.34 kilograms in weight as determined by 10-day metabolism experiments during which nearly nitrogen-free rations were fed*

[Totals are for 10-day experimental period]

Trial No.	Lamb No.[a]	Average weight	Nitrogen in feed consumed	Fiber in feed fed	Total urinary nitrogen	Total fecal nitrogen	Total of urinary and fecal nitrogen	Protein [b] per 1,000 pounds live weight
		Kilograms	*Per cent*	*Per cent*	*Grams*	*Grams*	*Grams*	*Pounds*
5	1 e	28.24	None.	15.99	12.38	12.85	25.23	5.62
	3 w	30.73	0.11	15.99	9.58	19.57	29.15	5.93
	5 e	20.07	.11	15.99	7.78	12.29	20.07	6.25
6	2 e	22.34	.10	15.99	5.90	12.14	18.04	5.05
	4 w	29.37	.07	15.99	8.61	17.62	26.23	5.58
	6 w	22.34	.12	15.99	4.33	13.38	17.71	4.95
15	1 e	25.17	.11	13.30	10.54	18.30	28.84	7.16
	3 w	28.58	.12	13.10	8.58	26.29	34.87	7.62
	5 e	19.28	.10	12.83	6.00	20.15	26.15	8.48
16	2 e	20.07	.11	15.07	4.15	20.34	24.49	7.62
	4 w	26.20	.14	15.07	9.58	18.00	27.58	6.58
	6 w	19.73	.13	15.07	9.45	18.52	27.97	8.86
Average		24.34	.11	15.03	8.07	17.45	25.53	6.64
Average per day					.807	1.745	2.553	.664

[a] Letters e and w indicate ewe and wether respectively wherever used after sheep number in all tables.
[b] To be supplied in net-protein form.

Robison (*12*) points out that starch prepared in the commercial way contains some nitrogen and that its value to the body is not known. If the value of this nitrogen in the nearly nitrogen-free diet is zero, then the real nitrogen requirements will be less than that computed from the output by the full amount of such a nitrogen intake, since the latter must be excreted in addition to the nitrogen that results from the catabolism of body protein. If, on the other hand, the value of this nitrogen for replacement or increase of body nitrogen is 100 per cent, then it will spare or produce an equal amount of body nitrogen, and the observed output during feeding of the nitrogen-free ration will represent the actual minimum requirement. If, however, the value of

this nitrogen for replacement or increase of body nitrogen is 75 per cent, then the other 25 per cent appears in the excreta, and the excretory nitrogen should be reduced by this amount.

The average dry-matter intake per lamb during the 12 nearly nitrogen-free ration periods was 273.8 gm. daily. With an 0.11 per cent average nitrogen content, this means an average daily intake of 0.301 gm. of nitrogen. If this nitrogen has a value of zero, then this quantity should be subtracted from the 2.554 gm., which is the average total nitrogen excretion from both the feces and urine. If this is done the maintenance requirement of the lambs is lowered by almost 12 per cent.

The data in Table 1, in addition to the dry-matter intake, were used in computing the fecal nitrogen per gram of dry matter ingested and the urinary nitrogen per 100 gm. of body weight per day. Tables 2 and 3 contain these data arranged according to trial and according to lamb, with necessary averages which are used in computing the biological values of the proteins in rations fed during intervening periods.

TABLE 2.—*Fecal nitrogen per gram of dry matter ingested and urinary nitrogen per 100 grams body weight, based on trials during which nearly nitrogen-free rations were fed*

[Totals are for 10-day experimental periods]

Trial No.	Lamb No.	Average weight	Dry matter ingested	Total fecal nitrogen	Total urinary nitrogen	Fecal nitrogen per gram dry matter ingested	Urinary nitrogen per 100 grams body weight per day
		Kilograms	*Grams*	*Grams*	*Grams*	*Gram*	*Gram*
5	1 e	28.24	1,502	12.85	12.38	0.0086	0.0438
	3 w	30.73	4,155	19.57	9.58	.0047	.0312
	5 e	20.07	2,176	12.29	7.78	.0057	.0388
6	2 e	22.34	1,868	12.14	5.90	.0065	.0264
	4 w	29.37	2,908	17.62	8.61	.0061	.0293
	6 w	22.34	2,341	13.38	4.33	.0057	.0194
15	1 e	25.17	2,924	18.30	10.54	.0063	.0419
	3 w	28.58	3,736	26.29	8.58	.0070	.0300
	5 e	19.28	2,441	20.15	6.00	.0083	.0311
16	2 e	20.07	2,862	20.34	4.15	.0071	.0207
	4 w	26.20	2,722	18.00	9.58	.0066	.0366
	6 w	19.73	3,218	18.52	9.45	.0058	.0479

TABLE 3.—*Fecal nitrogen per gram dry matter ingested and urinary nitrogen per 100 grams body weight, based on trials during which nearly nitrogen-free rations were fed*

Lamb No.	Trial No.	Fecal nitrogen per gram dry matter	Urinary nitrogen per 100 grams body weight	Average fecal nitrogen per gram dry matter	Average urinary nitrogen per 100 grams body weight
		Gram	*Gram*	*Gram*	*Gram*
1	5	0.0086	0.0438	0.0075	0.0428
	15	.0063	.0419		
2	6	.0065	.0264	.0068	.0236
	16	.0071	.0207		
3	5	.0047	.0312	.0059	.0306
	15	.0070	.0300		
4	6	.0061	.0293	.0064	.0330
	16	.0066	.0366		
5	5	.0057	.0388	.0070	.0359
	15	.0083	.0311		
6	6	.0057	.0194	.0058	.0337
	16	.0058	.0479		

The make-up of the nearly nitrogen-free rations is reported in Table 1 and 5. Their content of the various feeds on a percentage basis is as follows:

Feeds:	Trial Nos. 5 and 6 (per cent)	Trial Nos. 15 and 16 (per cent)
Straw [5]	51. 5	47. 1
Starch [6]	31. 0	25. 7
Sugar	16. 0	25. 7
Oil	1. 5	1. 5
Total	100. 0	100. 0

During trials 15 and 16 the percentage of sugar was slightly increased and the starch and straw percentages increased. The changes resulted in a somewhat more palatable ration, and no nauseating effect accompanied the larger sugar intake.

At no time during the experimental periods were any digestive disturbances noticed, the lambs appearing normal in every way. The feces were extremely light in color, as might be expected from the make-up of the ration, but they were of normal consistency.

The lambs had free access to salt during all preliminary periods, and salt was daily fed by hand during experimental periods in quantities of one-fourth to one-third of an ounce according to the quantity (*4*) recommended by Kellner.

Precaution against eating of bedding straw during preliminary periods of feeding nitrogen-free rations seemed unnecessary since the straw used for bedding was the same kind as that used in the daily ration. During other periods the bedding was placed in burlap sacks.

The lambs were fed in separate pens, one lamb to a pen, during all preliminary periods, and were kept singly in the metabolism crates. Since all fleeces were carefully trimmed, including the dung tags, there was very little wool mixed with the feces during collection periods. Furthermore, close observation failed to show that the lambs at any time were eating their own wool.

Results previously obtained by the writer in nitrogen metabolism studies with lambs, together with results reported by other investigators for man and several species of the lower animals, are shown in Table 4. The total urinary nitrogen of lambs, per kilogram of live weight per day (0.0331 kgm.) is strikingly similar to that of man. The urinary nitrogen of the albino rat and the dog are seven and five times greater, respectively, than that of the lamb.

[5] Selected stems of ripe Albit wheat straw, very low in nitrogen, were used.

[6] Buffalo starch is made by the wet process of grinding and separating starch from corn and is very low in nitrogen. The starch containing gluten is settled on long tables, separating the gluten. The starch is then flushed from the tables, washed on vacuum filters with fresh water to substantially remove soluble matter, dried in kilns, and ground.

TABLE 4.—*A comparison of the urinary nitrogen excreted per kilogram of body weight on high energy nearly nitrogen-free rations for man and various species of animals* [a]

Observer and date	Animal	Weight	Ration	Total nitrogen in urine per kilogram live weight
		Kilograms		*Gram*
Folin (1905)	Man	85.7	Starch, cream, 1 gm. N	0.0420
Graham and Poulton (1912)	do	62.4	Starch, cream, 0.912 gm. N	.0445
Do	do	72.4	Starch, cream, 1.23 gm. N	.0498
af. Klercker (1907)	do	88.0	Low N	.0319
Robinson (1922)	do	60.5	Carbohydrate, fat, 0.3 gm. N	.0352
Do	do	58.0	do	.0355
McCollum (1911)	Pig	10.9	Carbohydrate	.0495
Do	do	68.4	do	.0387
Mendel and Rose (1911)	Rabbit	1.74	do	.1260
Murlin (1907, 2)	Dog	11.3	do	.1580
Mitchell (*6*) (1923)	White rat	.134	N-free	.2198
Sotola (1928)	Black-faced lamb	24.3	Nearly N-free	.0331

[a] With the exception of the last two lines, this table is a part reproduction of Robinson's Table 1 (*12*, *p. 115*).

DISCUSSION OF BIOLOGICAL VALUES

A complete report of metabolism data, upon which the computation of the biological values is based, is given in Table 5. Nearly nitrogen-free rations fed during trials 5 and 6, and 15 and 16, the beginning and end of the series, form the basis for determining the body nitrogen in the urine and feces during the intervening periods. On the basis of these trials, the fecal nitrogen per gram of dry matter consumed and the urinary nitrogen per 100 gm. of body weight were determined for each lamb. These data were then applied to the corresponding lambs during the intermediate periods of protein feeding, in computing the body nitrogen in the urine and feces, in proportion to a varying body weight and dry-matter intake.

The data for the nearly nitrogen-free rations are contained in Table 2 and rearranged according to lamb numbers in Table 3.

The results with lamb 1 (Table 3) show that during trial 5 the fecal nitrogen per gram of dry matter consumed was 0.0086 gm. and during trial 15, 0.0063 gm. These results can be averaged and the average applied to the intervening periods, or else the decrease can be applied in a linear fashion to the intervening periods. Both methods were used, and in nearly all instances little difference in the biological values can be detected. For comparison, these values computed by applying the results of the nearly nitrogen-free rations in a linear fashion, or by averaging them, may be found in the last two columns of Table 5. The former method (6) is given preference.

TABLE 5.—*Summary of metabolism experiments upon which the determination of the biological values of proteins in alfalfa hay, corn silage, sunflower silage, and these feeds fed in mixtures, is based*

[Totals are for 10-day experimental periods]

Ration	Trial No.	Sheep No.[a]	Body weight			Feed intake, dry matter	Nitrogen intake	Fecal nitrogen	Body nitrogen in feces[b]	Food nitrogen in feces	Absorbed nitrogen	Total nitrogen in urine	Body nitrogen in urine	Food nitrogen in urine	Food nitrogen retained	Biological value[c] of protein by—	
			Initial	Final	Average											Linear method	Method of averages
			Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*		
Linseed oil, sugar, starch, wheat straw.	5	1 e	27.90	28.58	28.24	1,502	None.	12.85	12.92			12.38					
		3 w	31.52	29.94	30.73	4,155	4.88	19.57	19.52			9.58					
		5 e	20.64	19.50	20.07	2,176	2.41	12.29	12.40			7.78					
Do	6	2 e	23.36	21.31	22.34	1,868	1.99	12.14	12.14			5.90					
		4 w	31.07	27.67	29.37	2,908	2.11	17.62	17.73			8.61					
		6 w	22.68	21.99	22.34	2,341	3.07	13.38	13.34			4.33					
Alfalfa hay	11	1 e	32.20	32.20	32.20	10,633	241.85	87.81	79.75	8.06	233.79	108.13	13.81	94.32	139.47	59	60
		3 w	31.30	33.11	32.20	9,953	222.90	77.85	57.73	20.12	202.78	105.22	9.85	95.37	107.41	52	53
		5 e	23.13	24.04	23.59	7,502	172.63	58.91	52.51	6.40	166.23	81.10	8.26	72.84	93.39	56	56
Do	12	2 e	28.58	29.94	29.26	10,099	230.64	70.03	68.67	1.36	229.28	81.62	6.91	74.71	154.57	67	67
		4 w	33.79	34.47	34.13	10,411	233.27	70.04	65.59	4.45	228.82	138.73	11.26	127.47	101.35	44	45
		6 w	24.95	24.49	24.72	7,808	174.95	48.67	44.51	4.16	170.79	80.27	9.09	71.18	99.61	58	59
Sunflower silage	7	1 e	27.22	25.86	26.54	3,644	40.87	30.05	29.88	.17	40.70	17.36	11.54	5.82	34.88	85	84
		3 w	29.03	27.22	28.12	3,644	40.87	29.31	18.58	10.73	30.14	21.36	8.72	12.64	17.50	58	61
		5 e	19.50	19.50	19.50	3,459	40.34	26.99	21.10	5.89	34.45	13.02	7.31	5.71	28.74	83	84
Do	8	2 e	22.68	23.13	22.91	3,522	39.94	24.56	23.25	1.31	38.63	14.56	5.84	8.72	29.91	77	77
		4 w	29.26	26.99	28.12	2,445	27.36	19.68	15.16	4.52	22.84	24.61	8.58	16.03	6.81	29	34
		6 w	21.55	21.77	21.66	3,706	40.05	26.26	21.12	5.14	34.91	15.58	5.22	10.36	24.55	70	76

[a] e and w are used, respectively, to indicate ewe or wether.

[b] Whenever the figure for body nitrogen in the feces is larger than the figure for fecal nitrogen, the food nitrogen is assumed to be 100 per cent digestible.

[c] To compute the biological values of proteins, the body nitrogen in the feces and urine for any particular lamb during periods of protein feeding is computed from the fecal nitrogen per gram dry matter and from the average urinary nitrogen per 100 grams body weight as determined during periods of nearly nitrogen-free feeding for the same lamb. (5, 6, 15, and 16.) The biological values in the last column are based on the averages of the nearly nitrogen-free period. The values in the next to the last column are based on differences of values 5, 6, 15, and 16. These differences are applied in a linear fashion to the intervening trials. In all cases the results with a certain lamb are applied throughout to all experiments with that animal.

TABLE 5.—*Summary of metabolism experiments upon which the determination of the biological values of proteins in alfalfa hay, corn silage, sunflower silage, and these feeds fed in mixtures, is based*—Continued

[Totals are for 10-day experimental periods]

Ration	Trial No.	Sheep No.	Body weight			Feed intake, dry matter	Nitrogen intake	Fecal nitrogen	Body nitrogen in feces	Food nitrogen in feces	Absorbed nitrogen	Total nitrogen in urine	Body nitrogen in urine	Food nitrogen in urine	Food nitrogen retained	Biological value of protein by—	
			Initial	Final	Average											Linear method	Method of averages
			Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*		
1 part alfalfa hay, 3 parts sunflower silage.	9	1 e	27.90	27.67	27.78	5,944	103.30	41.17	46.36	.....	103.30	55.63	12.00	43.63	59.67	57	58
		3 w	29.26	29.48	29.37	5,998	103.66	40.63	32.99	7.64	96.02	51.83	9.05	42.78	53.24	55	57
		5 e	21.55	21.55	21.55	5,670	101.40	33.11	37.42	.....	101.40	41.56	7.80	33.76	67.64	66	66
Do	10	2 e	25.40	26.08	25.74	6,126	104.81	49.32	41.04	8.28	96.53	34.46	6.31	28.15	68.38	70	71
		4 w	28.58	29.48	29.03	6,126	104.81	43.99	38.59	5.40	99.41	50.08	9.20	40.88	58.53	58	60
		6 w	23.13	24.27	23.70	6,126	104.81	37.09	34.92	2.17	102.64	39.23	6.80	32.43	70.21	68	70
Corn silage	13	1 e	33.24	33.57	33.45	6,685	55.22	43.41	47.46	.....	55.22	16.74	14.25	2.49	52.73	95	97
		3 w	33.79	34.47	34.13	6,746	55.64	41.12	41.83	.....	55.64	14.20	10.31	3.89	51.75	93	93
		5 e	24.95	24.49	24.72	4,608	38.10	29.65	34.10	.....	38.10	11.14	8.33	2.81	35.29	92	93
Do	14	2 e	29.48	29.03	29.26	6,507	60.67	45.40	44.90	.50	60.17	9.04	6.61	2.43	57.74	95	96
		4 w	33.57	36.29	34.93	8,168	75.17	50.40	52.28	.....	75.17	15.95	11.95	4.00	71.17	94	94
		6 w	27.69	28.12	27.90	7,002	64.81	41.79	40.61	1.18	63.63	15.26	10.60	4.66	58.97	92	91
1 part alfalfa hay, 3 parts corn silage.	13b	1 e	29.03	28.58	28.80	7,891	123.74	47.97	.....	.....	123.74	Lost	12.15	.....	.....	.....	.....
		3 w	34.93	34.93	34.93	10,521	164.99	29.16	69.44	.....	164.99	40.42	10.55	29.87	135.12	81	82
		5 e	26.53	25.86	26.20	7,014	109.99	15.43	55.41	.....	109.99	33.24	8.49	24.75	85.24	77	78
Do	14b	2 e	27.69	28.12	27.90	9,721	131.95	60.95	68.05	.....	131.95	20.77	6.05	14.72	117.23	88	89
		4 w	34.02	34.02	34.02	9,112	144.32	67.06	59.23	7.83	136.49	30.99	12.04	18.95	117.54	80	85
		6 w	26.31	25.86	26.08	7,680	98.96	49.07	44.54	4.53	94.43	27.03	11.14	15.89	78.54	83	80
Linseed oil sugar, starch, wheat straw.	15	1 e	26.31	24.04	25.17	2,924	3.67	18.50	.....	.....	.....	10.54	.....	.....	.....	.....	.....
		3 w	30.39	26.76	28.58	3,736	4.81	26.29	.....	.....	.....	8.58	.....	.....	.....	.....	.....
		5 e	20.87	17.69	19.28	2,441	3.06	20.15	.....	.....	.....	6.00	.....	.....	.....	.....	.....
Do	16	2 e	20.41	19.73	20.07	2,862	3.77	20.34	.....	.....	.....	4.15	.....	.....	.....	.....	.....
		4 w	26.53	25.86	26.20	2,722	4.26	18.00	.....	.....	.....	9.58	.....	.....	.....	.....	.....
		6 w	19.96	19.50	19.73	3,218	4.62	18.52	.....	.....	.....	9.45	.....	.....	.....	.....	.....

A study of the biological values in the last column of Table 5 shows some rather wide variations. For instance, in trial 7, during which sunflower silage was fed, the value obtained for lamb 3, a wether, was 61, whereas the values for the two ewe lambs in the same trial were 84 and 84. Similarly, in trial 8, where the same ration was used, a value of 34 was secured for a wether and values of 77 and 76 for a ewe and wether, respectively. Careful rechecking of the data fails to disclose any discrepancies. These differences must be due to a lower utilization of the proteins by the lambs during these 10-day periods, for the records show that these lambs were normal in every respect while the trials referred to were in progress.

Similarly, in trials 10, 8, and 12, low results were secured with wether lamb 4. However, in subsequent trials the results with this lamb appear to compare fairly well with those for others in the same group.

SUPPLEMENTARY EFFECTS OF PROTEINS FED IN MIXTURES

It has been pointed out that the biological value of a single protein is determined by its amino acid constitution, and that the amino acid present in the minimum quantity, or perhaps its absence, limits the usefulness of that particular protein for production, particularly if the amino acid that is lacking is one which can not be synthesized by the animal body and one which is needed in large amounts for that particular type of production. Adding another protein especially high in the missing amino acid generally results in a biological value of the mixture higher than a mathematical average based on the percentage of nitrogen that each protein contributes to the mixture. This is due to the supplementing effect of the added protein.

In this study alfalfa hay was fed alone and its biological value determined. Similar data were secured for corn silage and sunflower silage. Then alfalfa was fed in the proportion of 1 part to 3 parts of one of the two silages.[7] The biological values of the single feeds as well as of the mixtures were determined. The results are contained in Table 6.

It is very evident that the dry-matter intake during periods of nearly nitrogen-free rations is, with one exception, lower than during the intervening periods. (Table 5.) This is largely due to the unpalatable nature of this ration. For ideal comparisons it would have been desirable to secure a larger dry-matter intake. However, the rations during trials 5, 6, 15, and 16 contained starch, sugar, and oil, and were much higher in energy per gram of dry matter than the roughage rations fed during intervening trials. It was more important to secure an intake of energy well in excess of the net energy required for maintenance than to increase the dry-matter intake. This was accomplished.

The percentage of crude fiber in the nearly nitrogen-free rations approximates that of periods 9, 10, 13b, and 14b, upon which is based the conclusion of a supplementary effect between alfalfa and corn-silage proteins. This excludes any error that might be due to differences in crude-fiber content of the rations.

[7] These proportions were chosen because they appear to be those commonly used under practical conditions where silage is readily available.

TABLE 6.—*The biological values of proteins of alfalfa hay, corn silage, and sunflower silage, when fed to lambs singly and in mixtures*

Ration	Trial No.	Lamb No.	Dry matter consumed in 10 days	Protein in feed consumed	Fiber in feed consumed	Observed biological value [a]	Biological values—			
							Calculated	Average observed	Average calculated	Difference
			Grams	*Per cent*	*Per cent*					
Nearly nitrogen free [b]	5	1 e	1,502	None.	[c] 15.99					
		3 w	4,155	0.66	[c] 15.99					
		5 e	2,176	.66	[c] 15.99					
Do	6	2 e	1,868	.63	[c] 15.99					
		4 w	2,908	.41	[c] 15.99					
		6 w	2,341	.75	[c] 15.99					
Sunflower silage	7	1 e	3,644	1.52	7.13	85				
		3 w	3,644	1.52	7.13	58				
		5 e	3,459	1.54	6.78	83				
Do	8	2 e	3,522	1.55	6.03	77		67		
		4 w	2,445	1.53	5.34	29				
		6 w	3,706	1.52	6.44	70				
1 part alfalfa hay, 3 parts sunflower silage.	9	1 e	5,944	4.38	12.09	57	66			
		3 w	5,998	4.34	12.03	55	53			
		5 e	5,670	4.50	12.46	66	63			
Do	10	2 e	6,126	4.37	12.18	70	70	62	58	4
		4 w	6,126	4.37	12.18	58	38			
		6 w	6,126	4.37	12.18	68	61			
Alfalfa hay	11	1 e	10,632	12.63	34.20	59				
		3 w	9,953	12.31	34.50	52				
		5 e	7,502	12.75	34.12	56				
Do	12	2 e	10,099	12.38	34.33	67		56		
		4 w	10,411	12.13	34.57	44				
		6 w	7,808	12.13	34.57	58				
Corn silage	13	1 e	6,685	1.61	5.57	95				
		3 w	6,746	1.61	5.64	93				
		5 e	4,608	1.67	5.74	92				
Do	14	2 e	6,507	1.68	6.06	95		94		
		4 w	8,168	1.68	6.30	94				
		6 w	7,002	1.68	6.19	92				
1 part alfalfa hay, 3 parts corn silage.	13	1 e	7,891	4.29	13.05					
		3 w	10,521	4.29	13.65	81	62			
		5 e	7,014	4.29	13.12	77	65			
Do	14	2 e	9,721	4.29	12.21	88	74	81	64	17
		4 w	9,112	4.29	12.21	80	55			
		6 w	7,680	4.29	12.38	83	67			
Nearly nitrogen free [b]	15	1 e	2,924	.70	[c] 13.30					
		3 w	3,736	.72	[c] 15.10					
		5 e	2,441	.64	[c] 12.83					
Do	16	2 e	2,862	.68	[c] 15.07					
		4 w	2,722	.87	[c] 15.07					
		6 w	3,218	.83	[c] 15.07					

[a] Computed by the linear method; see footnote [b] Table 5.

[b] These rations consisted of raw linseed oil, cornstarch, cane sugar, and selected stems of Albit wheat straw.

[c] Percentage of fiber in ration fed.

The full data summarized in Table 6 show the biological values of alfalfa hay, corn silage, and sunflower silage when fed singly and in combination.

The silages made of corn and sunflowers are so similar in composition that the daily rations of lambs fed solely these succulent feeds are almost identical in their protein and fiber content. This is brought out in Table 6. Because of this similarity the biological values of the proteins in these two roughage rations may be compared directly. An average value of 67 for sunflower silage as compared with 94 for corn silage shows the superiority of the proteins of corn silage over those of sunflower silage. If one very low value secured with sunflower silage is omitted from the average, a higher average of 75 is obtained.

Similarly, mixtures of 1 part alfalfa hay with 3 parts of either corn or sunflower silage have practically the same protein content and a very similar fiber content. The biological value for the proteins of the alfalfa-sunflower silage mixture is 62. A calculated average, based on the percentage of nitrogen that each protein contributes to the mixture, and by the use of biological values determined when these feeds are fed singly, gives a value of 58. Thus only a slight supplementary effect is noted.

However, in the case of the alfalfa hay-corn silage mixture, the observed average biological value is 81 as compared with 64 for the computed average. There is a marked supplementary effect in the case of this latter mixture. A distinct rise in value of the proteins in an alfalfa-corn silage mixture is noted, whereas no such effect was found in the alfalfa hay-sunflower silage mixture.

NET-PROTEIN VALUES

The loss of nitrogen sustained by an animal on a ration containing sufficient energy but free from protein and other forms of nitrogen is assumed to be the measure of the protein minimum for maintenance.

It can not be concluded that an equivalent amount of digestible protein will cover this requirement. In a similar manner, the protein content of added tissue or of milk secreted does not measure directly the quantity of digestible protein required for their elaboration. There is a wastage of digestible protein in supplying body needs, just as there is a wastage of available energy. The wastage of protein seems to be due to differences in the chemical structure of the feed protein and of the product being formed, as pointed out by Mitchell and Villegas (*10*).

A determination of food protein required for a specific purpose must not only be based upon the quantity of protein that the body needs, but also on the wastage of food protein in filling this need. The wastage of proteins differs among different food proteins, and a measure of the nutritive value of any protein must include not only wastage due to partial digestibility, but also the wastage of digestible material, representing amino acids, left over in feed protein conversion to body protein or its utilization in the maintenance of body tissue. These left-over amino acids, though available for energy or energy storage, can not be used for meeting the protein needs that arise from lack of the proper supplementary fragments.

The biological value of a protein seems at present to be the most useful means of measuring the nutritive value of digestible protein.

By the use of average percentages of total protein, average digestion coefficients, measuring the wastage of protein in digestion, and average biological values, measuring the wastage of protein in metabolism, it is possible to compute what Mitchell (*10*) calls the "net-protein" value of feeds. Rather than use the biological value of feeds determined singly in computing the net-protein values of mixtures as pointed out by Mitchell and Villegas (*10*) it appears that the better method is to use, as far as possible, the biological value as determined for a particular mixture in order to eliminate the error due to the supplementing effect of the proteins of one feed upon the protein of another. This method was employed in computing Table 7.

By the use of this method it is of course necessary to determine the biological values of proteins of feeds for the proportions in which the feeds are commonly fed. The difficulty with this method is that there are too many mixtures used in practice to determine values for all. However, it seems highly desirable to determine the values for those most commonly used.

TABLE 7.—*Net protein values of common roughages fed singly and in combination to lambs*

Roughage	Protein in feed consumed	Coefficient of apparent digestibility [a]	Digestible protein	Average biological value of digestible protein	Net protein content
	Per cent		*Per cent*		*Per cent*
Alfalfa hay (irrigated)	12.39	68	8.43	56	4.72
Corn silage (Windus white dent)	1.66	27	.45	94	.42
Sunflower silage (Mammoth Russian)	1.53	33	.50	67	.34
1 part alfalfa hay and 3 parts corn silage	4.29	[b] 66	2.17	81	1.75
1 part alfalfa hay and 3 parts sunflower silage	4.39	[b] 61	2.68	62	1.66

[a] Determined in this investigation; each figure is the average of six coefficients of apparent digestibility.
[b] These values have been determined for the mixtures of feeds in column 1 and are not mathematical averages. Weighted averages for the mixtures based on coefficients of apparent digestibility determined by feeding single feeds and weighted in proportion to the quantity of nitrogen each feed contributes to the mixture were computed. The figure for the alfalfa hay-corn silage mixture was 57, being lower by 9 points. For the alfalfa hay-sunflower silage mixture the value was 58, showing a difference of 3 points. These differences between computed and actual values are due to the beneficial effect one feed exerts upon another. It is greatest for the alfalfa hay-corn silage mixture.

An 80-pound lamb, according to Armsby (*1*), requires 0.044 of a pound of digestible protein for maintenance alone. This quantity of protein can be supplied in the net-protein form by the following quantities of feed: 0.94 pound alfalfa, 2.51 pounds of a 1 to 3 mixture of alfalfa hay and corn silage, or 2.65 pounds of a 1 to 3 mixture of alfalfa hay and sunflower silage. It would be impractical to attempt to supply the protein maintenance requirement of lambs by feeding either of the silages alone, since the bulk would make the ration prohibitive.

Thus, the net-protein value is used in a manner similar to that employed by Armsby (*1*) in his use of net-energy values.

An 80-pound lamb, according to the maintenance requirement determined in this paper (Table 1), requires 0.053 pound of digestible protein. To meet this requirement, 1.12 pounds of alfalfa hay, 3.03 pounds of a 1-to-3 mixture of alfalfa hay and corn silage, and 3.19 pounds of a 1-to-3 mixture of sunflower silage must be supplied.

Nevens (*11*) reports that when the corn grain was combined with cottonseed meal or with alfalfa hay the resulting utilization coefficients tended toward a mathematical mean of the utilization coefficients secured with feeding stuffs when fed alone, but were nearer that of the feed other than corn. He concludes that the corn-grain proteins and the alfalfa-hay proteins do not exert any supplementary effect upon each other. The proteins of the whole corn plant, including the stalk, leaf, husk, and kernels, when fed in connection with alfalfa hay, however, show a marked supplementary effect upon each other, as evidenced by the biological values of the proteins when fed singly and in combination, as reported in this paper. The mathematical average of the mixture is considerably below that actually observed.

EFFECT OF DIFFERENT ROUGHAGE RATIONS ON WATER CONSUMPTION AND ON THE URINARY AND FECAL EXCRETION OF LAMBS

The data collected during this investigation afford an excellent opportunity for studying the effect that certain feeds have upon water consumption and upon urinary and fecal excretion. For comparative purposes, results obtained with lambs fed pea and wheat straw, though not discussed in this paper, are included in Table 8 (trials 1 to 4).

Hart and Humphrey (*3*), working with dairy cows, showed that alfalfa hay has specific diuretic properties, and that its ingestion is generally followed by a marked rise in the output of urine. It is suggested that salts contained in the hay, or possibly specific organic substances, produce this effect.

Table 8 contains the results of eighteen 10-day metabolism experiments during which eight different rations were fed. A study of colums 5 and 6 shows that during trials 5 to 8, 13 to 16, and 18, on an average 32 per cent of the total excretory nitrogen was found in the urine. These trials include the nearby nitrogen-free rations, the straight corn and sunflower silage rations, and a mixture of alfalfa hay and corn silage (trial 18). It forms the only exception in that the ration contained some alfalfa hay and still the urinary nitrogen comprised less than one-half of the total excreted.

The average urinary nitrogen is approximatley 59 per cent of the total excretory nitrogen for the groups fed alfalfa hay alone, while a ration of pea straw (Alaska) and one of wheat straw (Albit) alone show a distribution of 49 and 52 per cent. Apparently the partition of ingested nitrogen between the urine and feces is governed to a great extent by the digestibility and percentage of nitrogen in the ration.

Column 13, trials 7, 8, 13, and 14 shows that lambs fed sunflower silage and corn silage alone excreted 3.5 and 0.9 c. c. of urine, respectively, per gram of dry matter consumed. The lambs on sunflower and corn silage rations, drank 1.20 and 0.75 gm. of water per gram of dry matter. This shows strikingly the diuretic effect of sunflower silage.

Column 7 shows the quantity of water that the lambs drank when fed various rations. The data show that when sunflower silage and corn silage alone were fed the water consumption was reduced to a minimum. The averages 191, 158, and 245 gm. of water per 100 gm. of pea straw, wheat straw, and alfalfa hay, shows that the higher protein roughages stimulate thirst. Other factors determining the water consumption of lambs are temperature and humidity. The salt consumption was the same for all lambs, so this factor may be disregarded.

TABLE 8.—*Summary of water consumption, and feces and urine excreted by lambs as related to the type of ration fed*

[Totals are for 10-day experimental period and per head]

Trial No.	Ration	Lamb No.	Total food eaten	Total dry matter consumed	Total water consumed	Water consumed per gram of food	Total feces	Feces per 100 grams food consumed	Feces per grams dry matter consumed	Total volume of urine	Urine per gram of food consumed	Urine per gram of dry matter consumed	Nitrogen in urine	Nitrogen in feces
			Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*C. c.*	*C. c.*	*C. c.*	*Per cent*	*Per cent*
1	Pea straw	1 e	4,520	4,519	9,939	2.02	6,718	136.54	1.49	6,500	1.32	1.44	48.26	51.74
		5 w	3,955	3,715	7,416	1.88	4,418	111.71	1.19	8,500	2.15	2.29	51.33	48.67
		3 e	5,754	5,171	9,872	1.72	5,328	92.60	1.03	5,500	.96	1.06	52.99	47.01
2	do	2 e	4,245	3,997	9,986	2.35	4,770	112.37	1.19	7,000	1.65	1.75	51.98	48.02
		4 w	6,000	5,362	10,102	1.68	7,900	131.67	1.47	9,300	1.55	1.73	47.54	52.46
		6 w	5,151	4,702	9,340	1.81	6,967	135.26	1.48	10,000	1.94	2.13	39.90	60.10
3	Wheat straw	1 e	2,524	2,193	5,260	2.08	2,824	111.89	1.29	9,500	3.76	4.33	66.50	33.50
		3 w	3,816	3,284	6,666	1.75	5,787	151.65	1.76	3,500	.92	1.07	45.27	54.73
		5 e	2,593	2,263	3,381	1.30	3,730	143.85	1.65	5,500	2.12	2.43	48.94	51.06
4	do	2 e	1,590	1,461	3,800	2.39	2,305	144.97	1.58	4,000	2.52	2.74	57.76	42.24
		4 w	3,596	3,121	3,881	1.08	4,349	120.94	1.39	5,000	1.39	1.60	51.84	48.15
		6 w	3,273	2,864	2,836	.87	3,620	110.60	1.26	3,000	.92	1.05	44.03	55.97
5	Oil, sugar, cornstarch, wheat straw.	1 e	1,614	1,502	2,808	1.74	2,962	183.52	1.97	7,000	4.33	4.66	49.07	50.93
		3 w	4,591	4,155	5,889	1.28	5,061	110.24	1.22	7,000	1.52	1.68	32.86	67.13
		5 e	2,284	2,176	4,819	2.11	2,724	119.26	1.25	5,500	2.41	2.53	38.76	61.24
6	do	2 e	1,960	1,868	3,460	1.74	2,503	125.78	1.34	2,500	1.26	1.34	32.70	67.29
		4 w	3,192	2,908	4,667	1.46	3,939	123.40	1.35	3,500	1.10	1.20	32.82	67.17
		6 w	2,548	2,341	3,034	1.19	3,751	147.21	1.60	3,500	1.37	1.50	24.45	75.55
7	Sunflower silage	1 e	16,800	3,644	3,127	.19	3,488	20.76	.96	12,000	.71	3.29	36.62	63.38
		3 w	16,800	3,644	5,878	.35	4,018	23.92	1.10	15,000	.89	4.12	42.16	57.84
		5 e	16,354	3,459	320	.02	2,921	17.86	.84	9,000	.55	2.60	32.54	67.46
8	do	2 e	16,085	3,522	7,771	.48	5,160	32.27	1.47	14,500	.90	4.12	37.22	62.78
		4 w	11,214	2,445	2,896	.26	2,350	20.96	.96	8,500	.76	3.48	55.57	44.43
		6 w	16,463	3,706	5,175	.31	3,060	18.77	.83	12,500	.76	3.37	37.24	62.76
9	1 part alfalfa, 3 parts sunflower silage.	1 e	14,794	5,944	16,975	1.15	6,144	41.53	1.03	19,000	1.28	3.20	57.47	42.53
		3 w	14,931	5,998	14,771	.99	5,273	35.32	.88	15,000	1.00	2.50	56.06	43.94
		5 e	14,087	5,670	8,936	.63	5,200	36.91	.92	10,000	.71	1.76	55.66	44.34

10	do	2 e	15,000	6,126	17,862	1.19	7,976	53.17	1.30	15,000	1.00	2.45	41.13	58.87
		4 w	15,000	6,126	13,126	.88	5,780	38.53	.94	13,000	.87	2.12	53.24	46.76
		6 w	15,000	6,126	14,714	.98	4,960	33.07	.81	14,000	.93	2.29	51.40	48.60
11	Alfalfa hay	1 e	11,602	10,632	34,041	3.09	15,533	141.18	1.46	12,850	1.17	1.21	55.19	44.81
		3 w	11,300	9,953	15,918	1.41	10,972	97.10	1.10	9,725	.86	.98	57.48	42.52
		5 e	8,480	7,502	16,060	1.89	6,386	75.31	.85	4,700	.55	.63	57.92	42.08
12	do	2 e	11,620	10,069	34,095	2.93	16,063	138.24	1.59	9,500	.82	.94	53.82	46.18
		4 w	12,000	10,411	35,112	2.93	13,036	108.63	1.25	14,000	1.17	1.34	66.45	33.55
		6 w	9,000	7,808	22,141	2.46	7,865	87.39	1.01	9,000	1.00	1.15	62.25	37.75
13	Corn silage	1 e	21,515	6,685	6,145	.29	5,680	26.40	.85	7,000	.33	1.05	27.83	72.17
		3 w	21,600	6,746	3,110	.14	4,409	20.41	.65	6,000	.28	.89	25.67	74.33
		5 e	14,892	4,608	1,621	.11	2,543	17.08	.55	5,000	.34	1.08	27.31	72.69
14	do	2 e	22,672	6,507	7,559	.33	6,679	29.46	1.03	4,700	.21	.72	16.61	83.39
		4 w	27,960	8,168	7,565	.27	5,554	19.86	.68	6,700	.24	.82	24.04	75.96
		6 w	24,155	7,002	3,332	.14	3,633	15.04	.52	6,200	.26	.89	26.75	73.25
15	Oil, sugar, cornstarch, wheat straw.	1 e	3,262	2,524	9,848	3.02	2,479	76.00	.85	3,500	1.07	1.20	36.55	63.45
		3 w	4,660	3,736	5,718	1.22	3,664	78.12	.98	3,000	.64	.80	24.61	75.39
		5 e	2,723	2,441	7,122	2.62	2,780	102.09	1.14	2,000	.73	.82	22.94	77.06
16	do	2 e	3,225	2,862	8,047	2.49	2,767	85.79	.97	6,000	1.86	2.10	16.95	83.05
		4 w	3,060	2,722	7,940	2.59	2,845	92.97	1.04	9,000	2.64	3.31	34.74	65.26
		6 w	3,613	3,218	6,729	1.86	2,538	70.24	.79	9,000	2.49	2.80	33.79	66.21
13b	1 part alfalfa, 3 parts corn silage	1 e	18,000	7,891	19,005	1.06	6,002	33.34	.76	10,400	.58	1.32		
		3 w	24,000	10,521	13,408	.56	6,198	25.82	.59	8,225	.34	.78	58.09	41.91
		5 e	16,000	7,014	8,112	.51	3,694	23.09	.53	4,000	.25	.57	68.30	31.70
14b	do	2 e	24,000	9,721	18,639	.78	5,269	21.95	.54	10,000	.42	1.03	25.42	74.58
		4 w	21,000	9,112	19,252	.42	5,234	24.92	.57	10,000	.48	1.10	31.61	68.39
		6 w	19,350	7,680	8,452	.44	3,294	17.02	.43	5,500	.28	.72	35.24	64.76

SUMMARY

Biological values of the proteins in alfalfa hay, corn silage, and sunflower silage were determined. These were found to be 56, 94, and 67, respectively.

A combination of 1 part alfalfa hay and 3 parts of corn silage had a value of 81, while the mathematical mean calculated on the basis of the nitrogen that each contributed to the mixture was 64. This shows a difference of 17, due to the favorable supplementing effect of the two proteins.

Similarly, a value of 62 was obtained for the mixture of proteins contained in 1 part of alfalfa hay and 3 parts of sunflower silage. The mathematical mean of 58 in comparison shows only a negligible supplementary effect.

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